ABSTRACT

Improved Recovery of Multiple Microbial Indicator Organisms from Source and Treated Drinking Waters by Optimized Hydraulic Modification to Hollow-fiber Ultrafilters

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Microbial contamination of drinking water through natural or intentional events continues to pose a public health risk. Contaminated environmental and drinking water samples generally have low concentrations of pathogenic microorganisms making a reliable, efficient, and robust method for concentrating them prior to isolation and detection a necessity. Currently accepted methods for concentrating microorganisms from large volume water samples are time consuming, costly, and not simultaneous for the recovery of all classes of microbial pathogens of concern. In contrast, hollow-fiber ultrafiltration has proven effective as well as inexpensive for concentrating multiple classes of microbial pathogens from water but has been limited by having long sample processing times. The objective of this study was to modify the hydraulic configuration of the hollow-fiber ultrafilter (HFUF) to determine the most efficient and least time consuming configuration for assessing the microbiological quality of water. In this study, microbial indicator organisms (*Escherichia coli* KO11, coliphage MS-2, and *Bacillus atrophaeus* spores) were spiked in 10 L source and treated drinking water samples and then multiple test conditions for hollow-fiber ultrafiltration were examined. . Hydraulic modifications made to the system were the use of modified endcaps to increase the cross-sectional area of water flow and the use of multiple ultrafilters for

increasing the filtering surface area within the system. There were no significant differences observed for microbial recoveries over the range of hydraulic modifications to the hollow-fiber ultrafilters. Microbial recoveries ranged from 21% to 185% in source waters and from 57% to 112% in treated drinking waters by any of the hydraulic modifications tested, however, there were significant reductions in processing times, with increases as great as 207% in the filtrate rate observed over the conventional hollow-fiber ultrafiltration system These hydraulic improvements to the ultrafiltration protocol are suggested for routine testing of water as well as for emergency response so that corrective action can be taken immediately, thereby resulting in greater measure of protection for public health.

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I. INTRODUCTION

Outbreaks of infectious diseases continue to threaten human life with no indication of elimination in the near future by current control measures. Since the formation of civilized communities and particularly following the development and potential for using pathogenic microorganisms as weapons against other societies, scientists have searched for effective and reliable treatment methods for pathogens in order to better protect public health. Later, public health scientists recognized the transmission routes for various pathogens which led to the quest to develop reliable methods for detecting low numbers of pathogens in environmental matrices, particularly in water. Even today, protection of public health continues to depend on key actions, including use of effective methods for microbial recovery from the environment, application of proven detection techniques, and risk modeling that allows for a quick response to potential microbial contamination events that can threaten society.

With the recognition that microbial pathogens are linked to human disease outbreaks responsible for loss of human life, an understanding of pathogen survival and routes of transmission in the environment, and knowledge of how to prevent disease outbreaks from occurring in the future became critical elements of paradigms for current public health practices. Several important pathogens for causing waterborne disease outbreaks on the CDC's Candidate Contaminant List are *E. coli* O157:H7, a bacterium commonly associated with the enteric illness that can potentially lead to death, *Norovirus*, which causes 90% of the

non-bacterial forms of acute gastroenteritis, and *Cryptiosporidium parvum*, a hardy gastrointestonal protezoan parasite that is zoonotic. These pathogens can contaminate water supplies from natural fecal sources or through unconventional means, such as purposeful introduction. Source water protection of surface and ground water is the first barrier for public health protection of most conventionally provided municipal drinking water supplies. Natural contamination of surface water supplies can occur through floods or other natural disasters that allow a resevoir to become contaminated from a fecal soure. Unconventional contamination events can occur when a pathogenic organism or organisms are introduced at a point in the municipal treatment train through a terrorist act in order to do knowingly attempt infliction of harm on society. Therefore, it is imperative that water providers are able to rapidly and effectively analyze water for potential pathogens so that necessary actions can be taken to best protect consumers.

In the United States, drinking water is generally provided through groundwater or through surface water sources. For surface water sources, reservoirs are one of the primary methods of securing and providing a clean and safe water supply for consumers. Reservoir water is commonly processed centrally, with treatment, testing to assess safety, and piped distribution to the population via distribution to households for use as drinking, cooking and cleaning water. Tests for quality are routinely conducted by the producer/distributor to verify that the water is safe for consumption.

Microbial testing by most municipal water providers relies on the use of microbial indicator organisms of microbial pathogens, as required in current regulations. However, some larger municipalities have the means and capability to test directly for a subset of the microbial pathogens considered of greatest importance. The methods for concentration,

isolation, and detection of microbial pathogens set forth by the U.S. Environmental Protection Agency are often expensive and typically only test for one class or a narrow range of pathogens. They are also time intensive which does not allow for rapid detection of multiple classes of microbial pathogens that may be present in the water supply. Hollow-fiber ultrafiltration, most commonly used for hemodialysis, and water treatment, offers has provided a potentially effective, robust, and reliable alternative method for concentrating multiple classes of microbial pathogens from large volume water samples.

Hollow-fiber ultrafilters have been used to concentrate viruses, bacteria, and protozoan parasites in large volume water samples and have the ability to efficiently concentrate all three classes of pathogens simultaneously (Morales-Morales, Vidal et al. 2003). Kalbfuss et al. demonstrated hollow fiber ultra-filtration to be a highly efficient method of concentrating influenza A virus when modules have a 750 kilo-dalton (kDa) cut-off (Kalbfuss, Genzel et al. 2007) while other studies document effectiveness in concentrating HIV viruses (Cruz, Martins et al. 1999) as well as porcine parvoviruses (Maranga, Rueda et al. 2002).

HFUFs have been used for the recovery of microorganisms from multiple types of water using different filters, eluting solutions, pre-treatments, and methods. Because HFUFs were adapted from other primary uses (i.e. removal of particles from water or hemodialysis), prior research has focused on determining their appropriateness for recovering microbes from large volumes water and the development of optimized techniques for this purpose. Test waters used in past studies include surface water from lakes and reservoirs, ground water from wells, finished drinking water, and reagent water. Multiple waters were tested to determine potential differences in microbial recovery attributable to physical and chemical quality of the water. The physical and chemical parameters measured in previous studies

were pH, turbidity, and total dissolved solids and researchers attempted to correlate them with recovery rates of bacteria, parasites, and viruses. From these past studies, average recovery rates varied from 33% to 86% depending on the microbe and type of water analyzed. Conclusions from this past work were that hollow-fiber ultrafiltration is an effective means for recovering multiple classes of microbial pathogens of public health importance for a variety of different water matrices.

The focus of the current research was to test the efficiency of hollow-fiber ultrafiltration for recovery of viruses, bacteria, and protozoa from multiple types of water, with the goal of high recovery efficiency at minimum sample processing time. This was accomplished by testing multiple hollow-fiber ultrafilters, multiple eluting solutions used to recover the microbes from the filters, hydraulic modifications of the ultrafilters to allow for a greater cross-sectional water flow to pass through the microtubules. The use of multiple HFUFs was attempted to further improve the method for more rapid detection of microorganisms present in water supplies.

II. RESEARCH OBJECTIVES

The overall objective of this research was to improve hollow-fiber ultrafiltration methods for the rapid and robust concentration of microbial indicator organisms in environmental water samples by examining hydraulic modifications to the HFUF system. Specific objectives of these experimental trials were:

- Investigate the effects of multiple test waters on microbial concentration by hollow-fiber ultrafiltration and any corresponding effects on sample processing time,
- Investigate the use of endcap and design modifications to the HFUF system for effects on concentration of microbes and processing time,
- Investigate multiple eluting solutions for effects on recovery of concentrated microbial indicators from the hollow-fiber ultrafiltration system,
- Investigate HFUF tubule design for effects on microbe recovery and processing time, and
- Investigate HFUF hydraulic design modifications and the use of multiple HFUF for effects on microbe recovery and processing time by the system.

III. LITERATURE REVIEW

A. Historical Significance of Microbes in Water

Microbial pathogens, with their ability to survive and be transmitted through water, continue to pose a problem for water providers and consumers worldwide. The potential for this public health problem can be traced throughout history to a time prior to our current understanding of the microscopic world and the concept of microbial pathogens as the source of waterborne disease outbreaks. With current advances in wastewater and water treatment technologies, the task of providing safe water to the world continues to be problematic and can affect both the developed as well as the developing world. In the developed world, there is routine monitoring of water supplies for detection of naturally occurring as well as intentionally introduced bioterrorist threats. Waterborne infectious disease continues to be of great public health significance today, with 127 drinking water outbreaks being reported to the CDC from 1990 to 1998 (Madigan, Martinko et al. 2006).

Microbial pathogens of concern today associated with source and finished drinking water supplies are diverse and include all classes of microorganisms. Those that are of most concern for the waterborne route of exposure are the enteric bacteria, viruses, and protozoa due to their pathogenicity, their stability in the environment, and their tolerance to commonly used drinking water disinfectants (Leclerc, Schwartzbrod et al. 2002). Enteric pathogens pose a significant threat for infections due to their ability to withstand the digestive process, remain viable, and infect the gastrointestinal tract of susceptible hosts. Presently, thirteen

pathogens have been identified as being of greatest concern for waterborne disease outbreaks in the United States and include *Giardia, Cryptosporidium,* Norovirus, *E coli* O157:H7, *Shigella, Campylobacter jejuni, Legionella, Salmonella,* non-typhoid, *V. cholerae,* Hepatitis A virus, *Naegleria fowleri, Plesiomonas shigelloides,* and *Yersinia* (Craun, Craun et al. 2006).

B. Monitoring Requirements for Microbial Contaminants in Water

Since the enactment of The Safe Drinking Water Act in 1974, the US EPA has implemented drinking water standards for monitoring water supplies to ensure safe levels of chemicals in finished drinking waters supplied to United States residents. In 1989, they began to monitor for microbial contamination as well with the enactment of The Total Coliform Rule and have since increased monitoring and regulation standards that are considered protective of public health. Currently the Long Term 2 Surface Water Treatment Rule, published in the Federal Register on January 5, 2006, is in place to regulate the number of disease causing microorganisms in drinking water. The methods that are published and currently being used for drinking water monitoring are broken into categories based on the type of microorganism being monitored, and include *Aeromonas*, coliphages, *Cryptosporidium*, *Escherichia coli (E. coli),* enterococci, *Giardia*, mold, total coliforms, and viruses.

Current regulations set by the Environmental Protection Agency have attempted to prevent inadvertent contamination of our drinking water supplies in an effort to thwart microbial outbreaks caused by exposure to fecally contaminated water. *Cryptosporidium parvum*, *Giardia lamblia*, and enteric viruses all have a Maximum Contaminant Level Goal (MCLG) of zero set by current EPA standards which are "The level of a contaminant in

drinking water below which there is no known or expected risk to health." The Maximum Cantaminant Level (MCL) also set by EPA standards however varies from organism to organism. *Cryptosporidium parvum* must be reduced by 99%, *Giardia lamblia* by 99.9%, and enteric viruses by 99.99%, according to current regulations for drinking water. A secondary list, known as the Drinking Water Contaminant Candidate List (CCL), has also been developed and routinely updated by the US EPA for contaminants "which, at the time of publication, are not subject to any proposed or promulgated national primary drinking water regulation (NPDWR), are known or anticipated to occur in public water systems, and may require regulations under Safe Drinking Water Act (SDWA)." This list includes both *Adenoviruses* and *Noroviruses* for which there are no controls under current regulations.

C. Use of Indicator Organisms for Protection of Public Health

Indicator organisms have been used for over 100 years as a surrogate measure for pathogenic microorganisms and have proven to be useful for a variety of applications. Indicator organisms are typically non-pathogenic organisms associated with the digestive tracts of warm-blooded mammals and superficially resemble the enteric pathogens of public health concern. Because these indicator organisms are associated with the gut flora of warm-blooded mammals, they tend to be indicative of fecal contamination of the sample being tested. Currently, total coliform bacteria are used as drinking water standards with *E. coli* being the definitive member to indicate fecal contamination. *E. coli* is a bacteria within the phylum Proteobacteria, is gram negative, oblong, and relatively easy to culture. *Bacillus atrophaeus*, a gram positive bacterium of the phylum *Firmicutes*, is a sporulating bacterium that can form endospores under stressful environmental conditions which can remain viable

for long periods of time and which have been proposed as potential indicators of protozoan parasites.

In addition to the bacterial indicators, bacteriopages have been proposed as potentially useful indicators of human enteric viruses. Coliphages are a type of bacteriophage that specifically infects *E. coli* bacteria. One prototype strain, bacteriophage MS-2, is a single-stranded, male-specific RNA coliphage of the family *Leviviridae*, is made up of a relatively small genome of approximately 3,569 nucleotides (Madigan, Martinko et al. 2006), and was the first genome to be sequenced in 1976. Male-specific coliphages are used as viral indicators because they more closely resemble viruses of public health importance with regards to size and morphological characteristics.

D. Concentration Methods for Microbial Pathogens from Water

Some microorganisms are typically present in low concentrations in environmental waters and need to be concentrated to facilitate accurate and reliable isolation, detection, and quantification. This need for robust concentration of microbial indicator and pathogenic microorganisms in water has been addressed by research into filtration methodologies that can be applied to large volumes of water for increased sensitivity and greater protection of public health (Sobsey, Wallis et al. 1973). Generally, proposed concentration protocols consist of a primary step for concentrating microbial indicators and pathogens from large volume water samples, followed by secondary concentration and isolation of the organisms of interest, followed by the final step of detection and quantification. Secondary concentration of microorganisms using ultrafiltration (Divizia, De Filippis et al. 1989), precipitation using salts (Armon, Arella et al. 1988; Lewis and Metcalf 1988), organic

flocculation (Dahling and Wright 1986) and immuno-affinity concentration (Schwab, De Leon et al. 1996) have been successfully used to further concentrate microorganisms allowing for detection of low numbers of organisms in primary environmental concentrates.

E. History of Membrane Technology and Applications in the Water Industry

The first synthetic membranes were developed by Adolf Eugen Fick in 1865 (Smith and Hashemi 2004). In the 1960's, membrane technology changed remarkably with the evolution and development of synthetic asymmetric membranes by Sourirajan and Loeb at the University of California- Los Angeles (UCLA) (Sirkar and Lloyd 1988) .Since this breakthrough, membrane technology has been applied and used in many fields for both industrial as well as scientific purposes (Juliano, 1997). In the water industry, applications for filtration techniques are wide and diverse and include using low-pressure membranes and reverse osmosis for drinking water treatment, as well as membrane filtration and ultrafiltration for microbial concentration and isolation of microbial, physical, and chemical contaminants for onsite laboratory monitoring. Due to the size differences, viruses are generally concentrated through electrostatic interactions allowing for adsorption to and elution from the filter media (Simmons, 1995). Bacteria and parasites are concentrated by physical removal on the surface of membrane filters.

F. Negatively Charged Filters

Negatively charged filters have been successfully used to capture and concentrate microorganisms. Bacteria and protozoan parasites are generally retained on the surface of filters by physical exclusion based on the pore size of the filter and the size of the organism.

There are two measures for the pore size of filter media: nominal and absolute. Nominal pore size refers filters that have multiple size pores across the surface with an average value. Absolute pore size refers to filters with similar size pores that allow for absolute size particle retention.

Because viruses are very small, virus particles are generally concentrated using porous filter media through electrostatic interactions. This process utilizes the principal that virus particles are largely negatively charged at ambient environmental pHs and can be attracted to a negatively charged filter using a catatonic bridge. Disadvantages for using negatively charged filter media for concentration of viruses include their potential inactivation during concentration and the necessity to add chemicals for virus capture, typically a divalent salt. In order to streamline virus adsorption to filters, trivalent salts, including Aluminum Chloride ($AlCl_3$), have been used in an attempt to increase virus adsorption with a decreased amount of salt added to the water (Wallis, Henderson et al. 1972). Another method attempted to increase virus adsorption on charged filters was the use of filter pretreatment with Nalco catatonic polymers which showed increased rates of virus adsorption when compared to untreated filters (Preston, Vasudevan et al. 1988).

G. Charge Modified Filters for Virus Concentration

Electropositive filter media has been successfully used to concentrate viruses from large volume environmental water samples. The charge modified filters, designed such that the charge on the filter media is more positive as compared to the previously described negatively charged filter media, have been composed of various materials and chemical compounds (Simmons, 1995). Sobsey et al. showed cellulose "charge modified" resin

mixtures to have positive charges at pH 5 to 6 and demonstrated the utility of electropositive filters for adsorption of virus particles in water samples without the necessity of water pre-treatment prior to sample processing (Sobsey and Jones 1979). Following this leap in virus concentration methods, a simpler more reliable method was developed using positively charged disk filters for the detection of relatively small amounts of poliovirus from tap water (Sobsey and Glass 1980). Sobsey et al. later developed filtration methods for large volume water samples based on a pleated cartridge filter using the previously developed Cuno media called the Virozorb 1MDS and reported that this method could be used without modification to water samples that were previously needed for negatively charged filter media (Sobsey and Glass 1980). Following this breakthrough, the Virozorb 1MDS has been the primary filter used for the concentration of viruses from large volumes of source and treated water and is currently the only accepted filter for use by the US EPA for virus recovery from water. However, positively charged filters have several disadvantages which include the cost, interference of virus recovery by other negatively charged media, and potentially highly variable recoveries with emerging or re-emerging viruses of public health importance (due to the fact that the filter relies on electrostatic interactions and the isoelectric points of the viruses may be unknown). Humic materials and fulvic acid are found in natural waters and possess a charge close to that of the negatively charged viruses that has proven detrimental for virus adsorption to the positively charged filter media. Several studies have demonstrated the negative effects of natural organic materials on microbial concentrations using charge-modified filter media (Guttman-Bass and Catalano-Sherman 1985; Sobsey and Hickey 1985; Guttman-Bass and Catalano-Sherman 1986). Other studies have attempted to find alternatives to the charged filter media by modification of conventional filter technology with

the goal of reduce filter costs and simplification of the sample processing mythology (Ma, Naranjo et al. 1994).

H. Ultrafiltration

Ultrafiltration is a technique for concentrating particles that is defined as "filtration through a medium (as a semipermeable capillary wall) which allows small molecules (as of water) to pass but holds back larger ones" (Merriam-Webster Inc. 2005).The long and complex history of this method began in 1748 with the discovery of membrane filtration by Abbe Nollet. He observed that a more dilute silution would cross over a semipermiable membrane to a more concentrated solution. This discovery continued in 1894 when Graham used a semi-permiable membrane to separate a solution into its individual parts (Cheryan 1986). Membrane technology continued to evolve when Fick developed the first synthetic membranes from nitrocellulose (Cheryan 1986). Following this, Bechold developed techniques to regulate pore size in filters as well as to coin the term "ultrafiltration" (Cheryan 1986). One of the first applications of membrane technology was for the detection of pathogen size. Using filters essentially as a sieve, pathogens were passed through the filters at right angles to the pores and Poiseuilles Law was used to determine microbe size using the pore size of the filter membrane (Clifton, Schultz et al. 1931).

I. Ultrafilter Design and Construction

Ultrafiltration, as opposed to other filtration methods, is directly dependent on the flow of fluids through the ultrafilter as well as the particle size and shape in the filter. Ultrafiltration designs can be tubular (internal diameter (ID)>10mm), hollow fiber

(ID<1.3mm), plate, spiral wound or vortex flow. The tubular ultrafiltration design uses larger tubing ranging from 10 to 25 mm in diameter and can range from 2 to 20 feet long in parallel or in series. Tubular designed ultrafilters are used to separate larger particles based on the larger inner diameter of the filter material. Hollow-fiber ultrafilters are generally arranged as bundles ranging from 5 to14, 000 tubes with a denser skin inside each tube. The diameter of the tubing is usually less than 1.3 mm and operates through tangential flow, meaning the flow is tangential to the pores which allows for less clogging and better concentration of particles. Particles are not able to form a cake layer at the surface of the hollow-fiber membranes due to scouring effects as particles are swept through the filter at 90° to the flow of the filtrate through the pores. The plate design for ultrafiltration is arranged as a stack of plates, or flat sheets, which are separated by spacers. The flow of the fluid is generally from the bottom to the top as well as radially inward across the membranes surfaces. Similar to the plate design, the spiral wound ultrafiltration design utilizes two flat sheets which are placed together with spacers between them. One side is attached to a perforated center tube and the arrangement is rolled around this tube. Vortex flow controls the fluid flow in the system allowing for an increased flux across the membranes. This is achieved by pumping the fluid between two cylinders, the inner of which is porous with a membrane attached to it that rotates at greater than 3000 rpm (Zeman and Zydney 1996). The filtration process is also affected by the shape of the particles as compared to their mass with linear particles being retained to a greater extent than spherical particles (Zeman and Zydney 1996). Another way that flow in the ultrafilter affects the retention of particles is by recirculation. Recirculation of a sample through the hollow-fiber ultrafilter efficiently retains and concentrates all particles

larger than the Molecular Weight Cut Off (MWCO) of the filter in the hold-up volume of the ultrafilter assembly (Simmons, Sobsey et al. 2001).

Type, brand, and flow inside the hollow-fiber ultrafilter have proven to have a large impact on both the microbial recovery efficiency as well as on sample processing time. The flow within HFUFs is generally based on the construction of the filter and placement of the pores. The simplest flow used is that of linear flow, which tends to be tangential, based on the construction of the HFUF. This flow type has been used for processing pharmaceutical products and is often the first choice for industrial applications. Tangential flow ultrafiltration has been used to separate cells, cell debris, and other insoluble particulate matter, typically in the range 0.02 to 10 microns from growth medium (Akeprathumchai, Han et al. 2004). Cross flow microfiltration is another method employed in the use of the HFUF that results from recirculation of a portion of the water back to a central reservoir while allowing a portion of the water to be passed through the filter. This prevents the accumulation of a gel layer that can reduce water flow, microbe recovery, and filter life. The configuration of the HFUF also allows for precise control of pressure and flow rate. This allows for the regulation of filtration conditions allowing for a more precise collection of the product of interest (Olszewski, Winona et al. 2005). Another flow pattern utilized for HFUF is that of the helical flow. In this method, the flow is forced outward due to the radial velocity profile. The maximum velocity is shifted away from the center-line outward due to centrifugal force and at a high enough flow rate vortices result. For ultrafiltration and microfiltration, it was shown that helical hollow-fiber membrane performed at a higher capacity than those without vortices (Gehlert, Luque et al. 1998).

Standard construction materials used for the production of ultrafilters have been derived from many different polymers, homopolymers, copolymers and blends of the three (Cheryan 1986). Most membranes are currently constructed of polyvinylidene fluoride, polyacrylonitrile, or polysulfone, which are highly resistant to heat and chemical wear, possess low protein binding characteristics, and are generally reusable following decontamination (Olszewski, Winona et al. 2005). Polyvinylidene fluoride is a highly non-reactive pure thermoplastic fluoropolymer which is used in applications requiring high strength, purity, resistance to chemicals, and relatively low melting points. Polyacrylonitrile (PAN) is a fibrous rubbery organic polymer that is generally a mixture of monomers with the main ingredient being acrylonitrile. Polysulfone is a thermoplastic material that is very strong, rigid, transparent, and maintains these properties over a wide temperature range from -100°C to 150°C. It is also highly resistant to changes in chemical composition and is not affected by a pH shift in the range from 2 to 13. Additionally, it has a high resistance to temperature making it an excellent choice for hollow-fiber ultrafilters.

The MWCO is also an important parameter for HFUFs used to concentrate microorganisms from water. Microorganism size varies greatly with class. Human enteric viruses generally range in size from 20 to 300 nm, most enteric bacteria range in size from 0.5 μm to as much as 5.0 μm, and zoonotic protozoan parasites range in size from 4 μm to 50 μm. With such a large range of sizes for microbial contaminants, the HFUFs must have a smaller or comparable pore size, to effectively concentrate them from large volume water samples. The MWCO determines the size of the particle that is retained and what is allowed to pass through the filter (Simmons, Sobsey et al. 2001), with most hollow-fiber ultrafilters ranging from 1,000 MW to 1,000,000 MW, equivalent to 0.1 μm to 0.001 μm (Cheryan

1986). Ultrafilters generally do not have a uniform pore size, so they can be rated as either an absolute MWCO (AMWCO) filter, which is the absolute size of the pore in the filter, or nominal MWCO (NMWCO) filter, which is the molecular weight where 90% of the particles are rejected.

J. Applications for Ultrafiltration

Ultrafiltration has been used for a variety of applications, with medical applications of hemodialysis and hemofiltration being the most widely used (Baumann and Kokott 2000). Ultrafilters used primarily for medical applications function to filter impurities from blood and substitute for the function of kidneys in patients with renal disorders as well as for drug detoxification in overdose cases. For each of these applications, blood is passed through the filter using the heart as the pump while a dialyzate is passed on the other side allowing the solutes or contaminants to move from the blood to the dialyzate by diffusion in a process known as hemodialysis (Cheryan 1986). Hemofiltration occurs when a pump is used to apply a constant pressure to the ultrafilter which removes those particles smaller than the MWCO of the filter while retaining the large particles and contaminants. This allows for a higher transport from blood to dialyzate than dialyzate to blood which prevents backflow, a problem previously reported for hemodialysis (Soltys, Zydney et al. 2000).

Biotechnology and cell harvesting is another large scale application for hollow-fiber ultrafilters that has been used in recent history by the biotechnological industry. Recently, ultrafiltration has taken on many more applications including production of high quality water, tissue culture reactor systems, as well as harvesting of enzymes and microorganisms (Cheryan 1986). High purity water, an essential need in today's growing technological

culture, often uses ultrafiltration to produce this needed commodity instead of distillation. By using ultrafiltration, water can be processed to ensure that it is of a high quality with minimal concentrations of particles and other contaminants.

Ultrafilters are also being used today as tissue culture reactor systems. For this use, cells are grown on the inside of the hollow-fiber tubing while allowing a continual passage of growth media needed by the cells to pass through. The fibers act as a passage point for any gas mixtures that are produced as a byproduct of the cultures allowing for a self sustaining highly productive culture device.

Ultrafilters have also proven useful for harvesting enzymes and microorganisms from large volumes. Microorganisms can be removed from waters for the protection of residents in the area or the HFUF can be used as a production device to minimize losses experienced by older techniques (Klein, Mahlandt et al. 1971; Suttle, Chan et al. 1991). One example of this use is for the production of adenoviruses using a hollow-fiber ultrafilter. Typically pathogens are recovered from their vectors using long and tedious processes with low recoveries, but ultrafiltration methods have been devised to increase production that were rarely associated with previous methods (Subramanian, Altaras et al. 2005). Ultrafiltration has also been used to harvest enzymes from plants when other methods are too expensive or technically difficult (Schade, Thompson et al. 2003) that may inhibit reliable ripening processes (Cass, Schade et al. 2000). This is accomplished using similar techniques and processes previously described for dialysis patients.

K. Application of Hollow-fiber Ultrafiltration for Concentrating Microbes in Water

The main areas where ultrafiltration may have profound effects and limitless possibilities are in the filtration of potable drinking water supplies. The processes that are currently in place for conventional processing of drinking water are sometimes ineffective for removal of smaller microorganisms. Because of this, some utilities have begun to use membrane technology for medium and large scale treatment of municipal waters, which have proven extremely effective for pathogen removal. Although the development and implementation of such technology has made great strides, membrane integrity continues to pose problems in some systems. Furthermore, questions still remain as to how the particulate matter that is retained within these plants should be treated and disposed. In addition to the use of membranes for water treatment, researchers have also suggested the use HFUFs in concentration and testing for bacteria, viruses, and parasites in drinking water prior to distribution (Kuhn and Oshima 2001; Simmons, Sobsey et al. 2001; Morales-Morales, Vidal et al. 2003).

Two types of HFUFs are generally used for concentrating microorganisms from water and include those that are reusable and those that are disposable or single use. The reusable HFUFs are typically constructed of a material that can be disinfected (Simmons, Sobsey et al. 2001). All three of the materials mentioned previously are used to produce filters of both the reusable and single use type. The main difference between these two HFUF types is cost, with single use filters costing around $30 to $50 and reusable HFUFs ranging to $100 and more.

When samples are processed using either of the two HFUF types, water is recirculated through the HFUF, reducing the sample volume with each passage, until the hold-up volume of the hollow-fiber ultra filtration system is achieved. Following recirculation of water

samples resulting in retention of particles in the system, microorganisms are recovered using an eluting or re-suspension media which is circulated through the system until the holdup volume is again reached, generally resulting in a final volume of only a few hundred milliliters. This represents a concentration factor of 100 times or more (10 liters of initial sample concentrated to a 100 mL sample retentate). Previous studies have examined different recovery parameters for retained microorganisms from the HFUF, including different types and sources of waters (environmental source and finished drinking waters), different eluting solutions, and different concentration parameters (different HFUFs, pump pressures, and restriction of backpressure on the HFUFs to increase flux)(Ebben, Meyer et al. 1981; Gohl, Raff et al. 1986; Eloot, De Wachter et al. 2002; Morales-Morales, Vidal et al. 2003). Standard eluting solutions that have been used to recover microorganisms from these filters are generally buffered solutions such as phosphate-buffered saline (PBS) supplemented with a surfactant such as Tween 80 or laureth-12 or some other defined substrate, such as glycine and glycine-NaOH (Simmons, Sobsey et al. 2001; Morales-Morales, Vidal et al. 2003). Other eluting solution additives have also been tested and have proven effective in the retrieval of microorganisms, primarily through the addition of Sodium Poly-Phosphate (NaPP) (Hill, Polaczyk et al. 2005).

Pretreatment of the HFUF prior to microbial concentration from water samples is another parameter that has been tested to determine any effects that it may have on recovery of microorganisms. Morales-Morales et. al investigated the recirculation of a 500 mL solution of 200 mg of sodium hypochlorite per liter for 30 minutes then adding a 0.1% sodium thiosulfate solution and re-circulating through the HFUF for 30 additional minutes (Morales-Morales, Vidal et al. 2003). Other researchers tested blocking the HFUF membrane using 5%

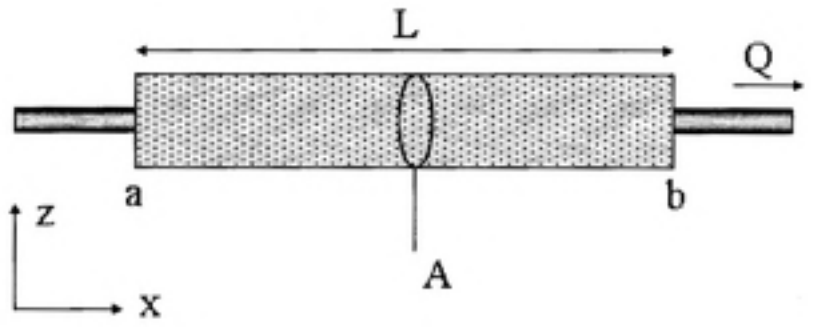
fetal bovine serum (FBS) followed by incubation at 20°C for 24 h. The FBS was then poured out prior to filtration and any unbound FBS was removed by flushing 20 liters of water through the HFUF (Kuhn and Oshima 2002). Another experiment tested 5% calf serum prior to filtration by re-circulating the solution across the surface of the filter for 1 hour on the day prior to water concentration followed by incubation with 200 mL 5% calf serum overnight at 4 °C on an orbital shaker (Olszewski, Winona et al. 2005). In all of the above experiments, there were no significant changes in the recovery of the microorganisms that could be attributed to pretreatment of the HFUF.

Sample size has also been a parameter tested in prior experiments where researchers have correlated microbial recoveries with the time required to concentrate water samples by hollow-fiber ultrafiltration. Sample size for prior experiments has ranged from 2 to 100 liters, with 10 liter samples being the volumes tested for most past experiments and for which most data has been collected (Kuhn and Oshima 2001; Kuhn and Oshima 2002; Morales-Morales, Vidal et al. 2003). The processing time for 10 liter volumes has depended primarily on the type of water sample being processed as well as the hollow-fiber ultrafilter being utilized. For surface water, generally containing more organic substances than ground waters, average processing time ranged from 90 minutes to 120 minutes depending on the HFUF tested, with those HFUFs utilizing tangential flow generally having longer processing times. Ground water sample processing times ranged between 20 and 30 minutes (Olszewski, Winona et al. 2005). Other experimental trials had an average processing time ranging from 30 and 45 minutes for 10 liter environmental water samples (Kuhn and Oshima 2002; Morales-Morales, Vidal et al. 2003).

L. Hydraulic Theory as Related to HFUF Setup and Microbial Concentration

Dynamic fluid flow is conventionally used in areas requiring the highest rate of efficiency to remove heat, organic content, or other materials from a system. It is commonly used in wastewater processing, heat transfer, as well as home use in air conditioners. The principals of flow within these systems and designs for their intended purposes are based upon fundamental laws of liquid hydraulics. The law most relevant to these experimental trials is Darcys Law (Figure 1) which states that total discharge, Q (units of volume per time, e.g., m^3/s) is equal to the product of the permeability (κ units of area, e.g. m^2) of the medium, the cross-sectional area (A) to flow, and the pressure drop ($P_b - P_a$), all divided by the dynamic viscosity μ (in SI units e.g. kg/(m·s) or Pa·s), and the length L the pressure drop is taking place over. It also assumes that greater the pressure gradient through the same formation material, the greater the discharge rate. The most common placements used for many of the processes requiring multiple exchangers of any fashion are parallel (Figure 2) and series (Figure 3) so that an increase in surface are can be obtained. Given this information, there are two potential ways in which flow through the system can be improved: either by increasing the cross-sectional surface area of the flow delivered to a unit or by increasing the surface area of the unit.

Figure 1. Darcys Law Illustration Showing Definitions and Directions (www.wikipedia.com)



$$Q = \frac{-\kappa A}{\mu} \frac{(P_b - P_a)}{L}$$

Figure 2. Series Flow Heat Exchange Hydraulic Design

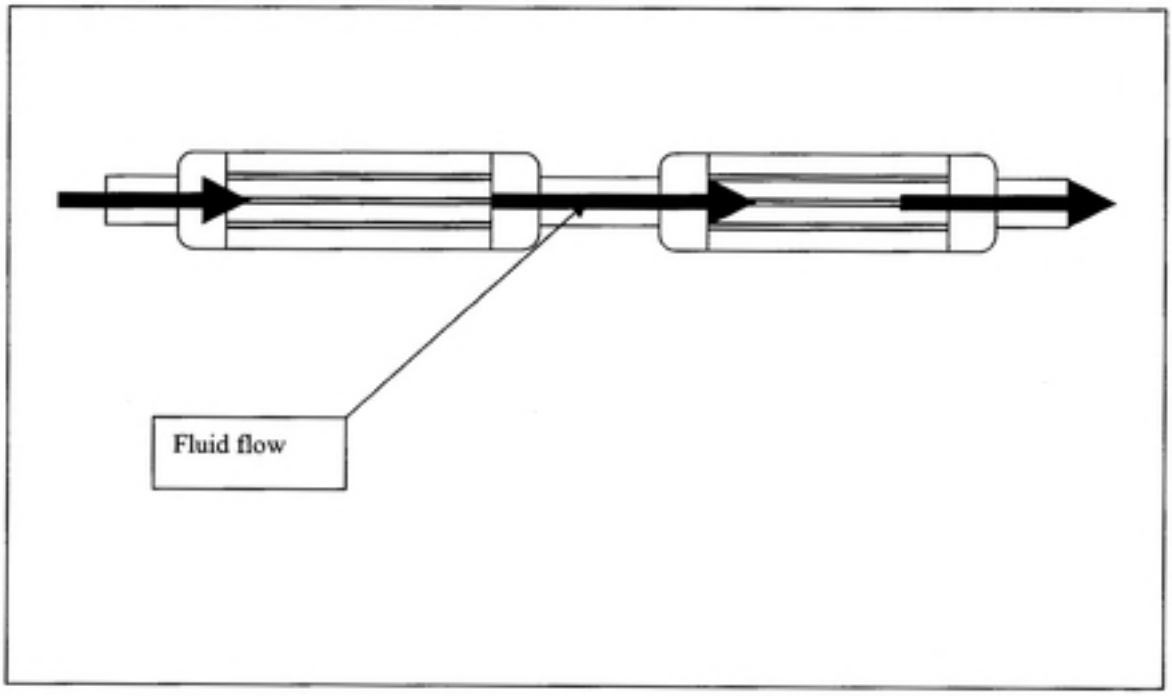
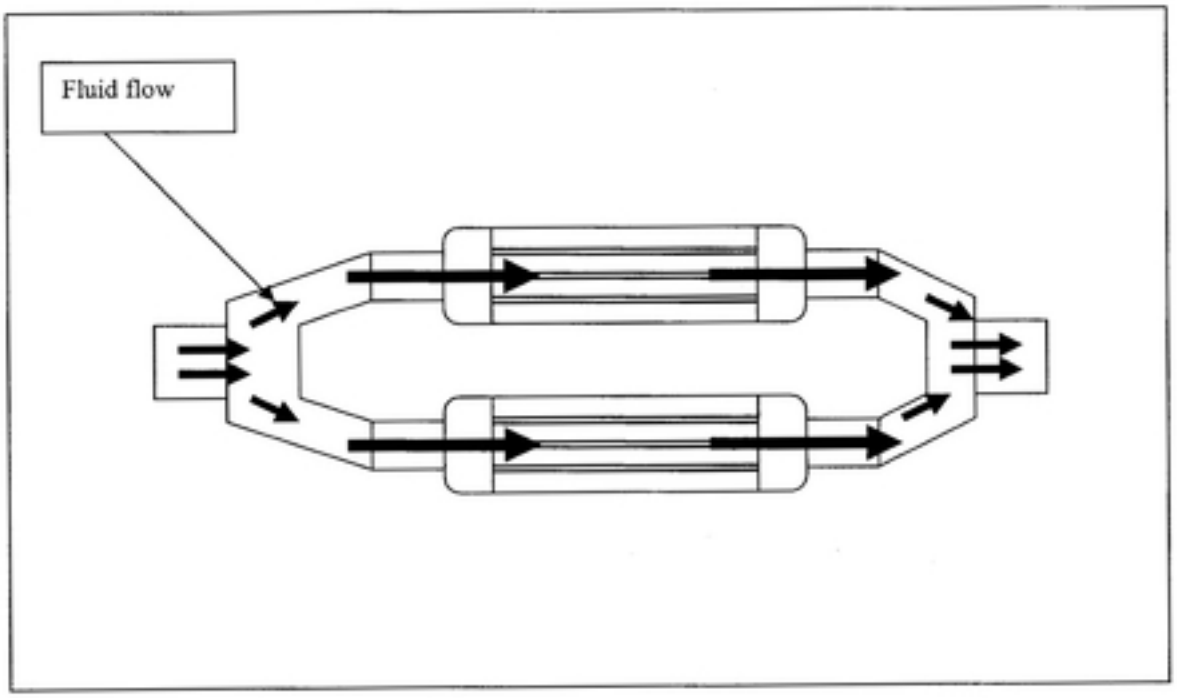


Figure 3. Parallel Flow Heat Exchange Hydraulic Design



IV. MATERIALS AND METHODS

A. Test Organisms

Escherichia coli KO11 (ATCC 55124), resistant to chloramphenicol, was used in these experiments in order to distinguish spiked from naturally occurring organisms in test waters. Cultures were propagated in tryptic soy broth (TSB) supplemented with chloramphenicol (1%) overnight in a shaker flask at 36°C. Bacterial stocks were supplemented with sterile glycerol (20%), aliquoted, and stored at -80°C until used for experiments. For experimental trials, a frozen aliquot was thawed, used for the experiment, and then discarded to minimize possible contamination of stocks used for experimental trials. For bacterial assay, serial ten-fold sample dilutions were spread onto pre-poured tryptic soy agar (TSA) plates supplemented with chloramphenicol (1%) in order to quantify only those spiked colonies of interest. Plates were inverted and incubated at 37°C overnight then enumerated the following day (20 to 24 hour incubation), with results expressed as colony forming units (CFU).

Coliphage MS-2 (ATCC #15597-B1) was obtained from American Type Culture Collection and was propagated and assayed using *E. coli* C-3000 (ATCC#15596) host bacteria. Virus stocks were prepared using a double agar layer (DAL) technique, where the bacterial host is combined the virus stock in 5 ml of 0.4% tryptone glucose yeast extract (TGYE) top agar at 45°C. The agar was mixed and distributed over prepoured 1.5% TGYE bottom agar plates and incubated upright at 36°C overnight. Plates showing confluent lysis

following incubation were supplemented with 5ml of sterile 1X phosphate-buffered saline (PBS) to loosen the top agar layer then removed to 50 ml conical centrifuge tubes. A half volume of Freon® (1, 1, 2-trichloro-1, 2, 2-triflourocthane) was added to each centrifuge tube, vortexed vigorously for two minutes, and then centrifuged at 1200x*g* for 15 minutes. The supernatant containing the virus stock was removed, aliquoted, and stored at -80°C until used for experiments. Virus assays were performed using the single agar layer plaque (SAL) method where agar is supplemented with log-phase host (*E. coli* C-3000), the virus stock or unknown sample dilution is added, mixed, poured onto a Petri dish. After the agar hardened, the plates were inverted and incubated overnight at 36°C with virus plaques enumerated the following day. Results were then expressed as plaque forming units (PFU).

Spores stocks of *Bacillus atrophaeus* were obtained from a commercial supplier (SGM Biotech, Inc., Bozeman, MT) and were stored at 4°C until used for experiments. To enumerate spore stocks and experimental trials, samples were serially ten-fold diluted, heat treated at 60°C for 10 minutes in order to inactivate vegetative bacteria, and spread onto pre-poured adenylate kinase 2 (AK2) bottom agar plates. Plates were inverted, incubated at 36°C overnight (20 to 24 hours), and enumerated the following day.

B. Experimental Test Waters

Waters used in these experimental trials were obtained from two different geographic locations, California and North Carolina. The California waters were obtained by the San Francisco Public Utilities Commission (SFPUC) from the Hetch Hetchy Reservoir, a manmade reservoir located in Yosemite National Park, and from the SFPUCs drinking water distribution system. New 10 L cubitainers (Cole – Parmer Vernon Hills, IL Catalog #A-

06100-30) were used to obtain and ship the samples to the UNC Microbiology Laboratory by overnight carrier. Cubitainers were used only once and then discarded to prevent possible cross contamination between samples. The SFPUC collected both source and treated drinking water from 10/30/2006 until 3/22/07. For the remainder of these experimental trials (9/10/2007 until 3/2/2008), 10 L volumes of source and treated drinking waters were collected as previously described by the Orange County Water and Sewage Authority (OWASA) located in Chapel Hill, NC. OWASA test waters were from the Cane Creek reservoir, located in Carrboro, NC, one of the two reservoirs from which OWASA uses as their source for drinking water. All test waters were analyzed for pH, turbidity, total chlorine, total coliforms, free chlorine, hardness and alkalinity. All sample data was recorded and statistically analyzed to determine if the waters were similar. Waters from each of the geographical region proved to be dissimilar, which is most likely due to the source of the water as well as the treatment train used by each utility for processing the waters. The majority (about 85%) of SFPUC treated water is not filtered due to their having a filtration exemption and was therefore significantly different from the treated waters from OWASA, which are conventionally treated by coagulation, flocculation, and filtration prior to disinfection. When statistically compared individually, each of the waters (SFPUC and OWASA) proved to be uniform with regards to measured chemical and physical parameters temporally over the duration of these trials. Source waters were also statistically different between geographical water sources, but when compared amongst samples drawn individually on different dates, there were no significance differences. For this reason, waters used for these trials were separated for comparisons based on their geographic origin.

Additionally, comparisons were made based on paired observations which allowed for internal control within each experimental trial.

Source or treated drinking water samples were used immediately for experiments or stored at 4°C for no more than 72 hours following their arrival at the UNC Microbiology Laboratory. Prior to experimental trials, treated drinking waters were dechlorinated using sodium thiosulfate (0.1g/L) with further confirmation of chlorine quenching by a commercially available Free and Total Chlorine Test Kit (Permachem® Reagents, Model CN-70) following the manufacturer's instructions. Waters were constantly mixed using autoclaved stir bars during experimental trials and new spigots were used to transfer test waters to the hollow-fiber ultra filtration systems being tested. A spiking procedure was used for the indicator organisms (*E. coli* KO11, coliphage MS-2, and *Bacillus atrophaeus*) that consisted of microbial stocks being added to sterile 50 mL conical centrifuge tubes as an intermediate dilution containing 30 mL of the sample water, which was then added to the 10 L test water volumes. Each of the 50 mL conical tubes were rinsed three times with test water sampled before spiking to ensure transfer of the microbial indicators to the bulk test waters. The 10 L bulk waters were mixed for a minimum of ten minutes to promote random distribution of the microbial indicators in the 10 L water volumes, and the waters were then used for experiment trials. Following this spiking procedure, initial water aliquots were removed in order to determine initial microbial titers. Following experimental trials, microbial titers in concentrated samples were compared to initial titers, corrected for volume differences, and method recovery efficiencies were computed.

C. Hollow-fiber Ultrafiltration for Concentrating Microbial Indicators from Water

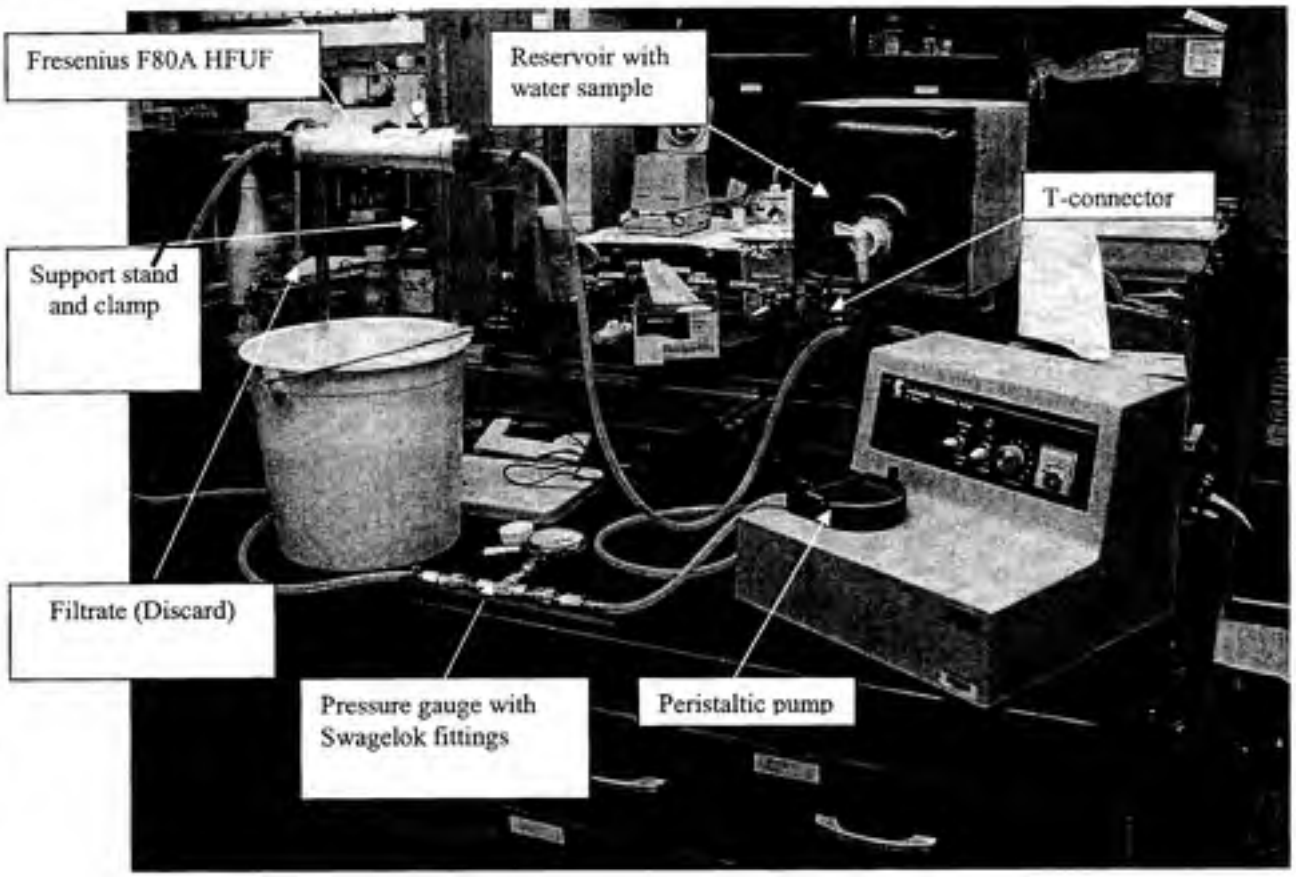
Hollow-fiber ultrafiltration for concentrating microbes from environmental waters is based on laminar flow recirculation of water through the system, which utilizes a peristaltic pump to recirculate the water from a reservoir through the ultrafilter then returned to the pump inlet (Figure 4). As the water is recirculated, particles larger than the molecular weight cutoff (MWCO) of the ultrafilter are retained as the retentate and the liquid portion is removed from the system as the filtrate. As the test water sample is recirculated, the retentate volume is reduced whereby upon completion, there is a 10 fold or greater increase in the concentration particles in the retentate. Because this system works by tangential flow, the liquid retentate, along with the particulate matter in the original sample allows for a constant scouring effect on the internal surfaces of the ultrafilter and tubing, thereby resulting in the absence of cake layer formation such as the case with conventional filters that prevents blockage of the micropores within the filtering system. Therefore, these tangential flow hollow-fiber ultrafiltration systems are capable of processing waters with heavy loads of particulate matter without becoming blocked.

In order to increase the flux of the system so as to increase sample filtrate rates resulting in reduced sample processing times, hydraulic modifications to the HFUF system are needed. The three modifications that were explored through these experiments were to test multiple HFUFs with different tubule designs which increase the surface area in the HFUF, to modify the HFUF endcaps, and to use multiple HFUFs in the system. Each of these modifications is based upon two of the variables within the Darcy's Law equation. By increasing the cross-sectional area of the endcaps, there is a greater volume of water that reaches the HFUF tubules which in turn, increases the volumetric flow rate through the filter, thus minimizing the time required to process a water sample. The use of alterative HFUFs with tubules that

increase the surface area and the use of multiple hollow-fiber ultrafilters in the system increases the filtering surface area of the system, which also increases the volumetric flow rate to the filter and in theory should reduce sampling time.

Following sample processing resulting in reduced retentate volume, an eluting solution is recirculated through the hollow-fiber ultra filtration system and concentrated particulate matter is recovered from the hold-up volume remaining. All laboratory equipment and solutions used in these trials were autoclaved and sterilized prior to experiments. All filters were obtained sterile from Fresenius Medical Care, used only once, and then discarded to prevent potential cross contamination. Tubing and modified end caps were autoclaved, washed, inspected for effects of the eluting solution, wrapped for sterility, and then re-autoclaved for sterilization for the next experiment.

Figure 4. Conventional HFUF Setup



1. Testing of Multiple HFUFs Based on Ultrafilter Design

Two ultrafilters were tested during the course of these trials and they were chosen based on the HFUF dialyzer design. In order that there was direct comparability, the two HFUFs were tested using similar setups, water types and microbial indicator organism concentrations. The HFUFs compared were manufactured by Fresenius Medical Care AG & Co. KGaA Bad Homburg, Germany and were the Hemoflow™ F80A and Optiflux® F200A. The primary differences between these two HFUFs are the flow pattern of the fluid through the filter. The Hemoflow™ F80A HFUFs have a parallel flow through the hollow-fibers (Figure 5), while the Fresenius Optiflux® F200A has a more turgid flow with a greater

surface area (Figure 6). Both of the HFUFs were similar in costs with the F80A costing $30 and the F200A over $100.

Figure 5. Fresenius Hemoflow™ F80A Flow Pattern and Tubing Setup

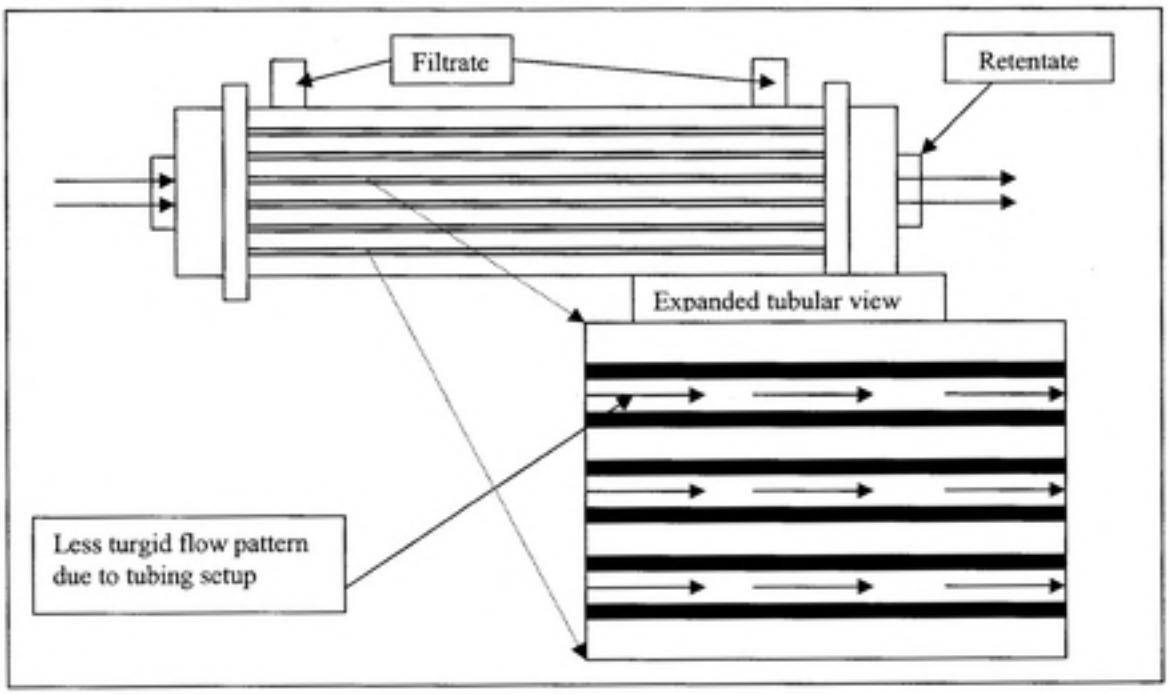
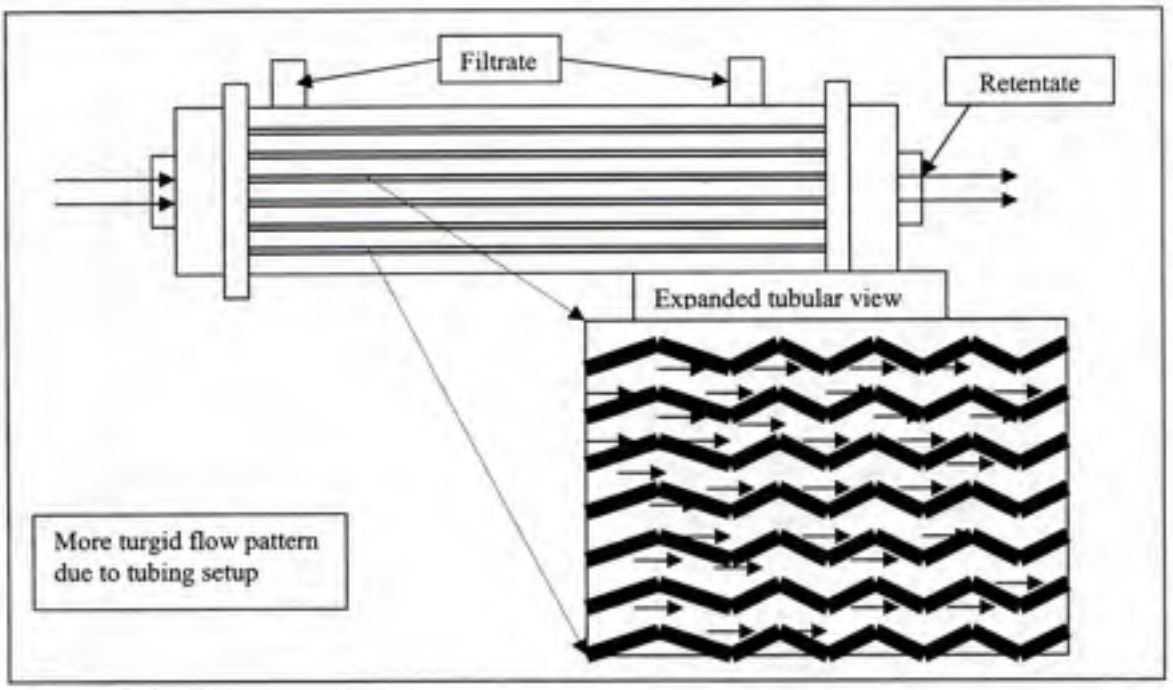


Figure 6. Fresenius Optiflux® F200A Flow Pattern and Tubing Setup

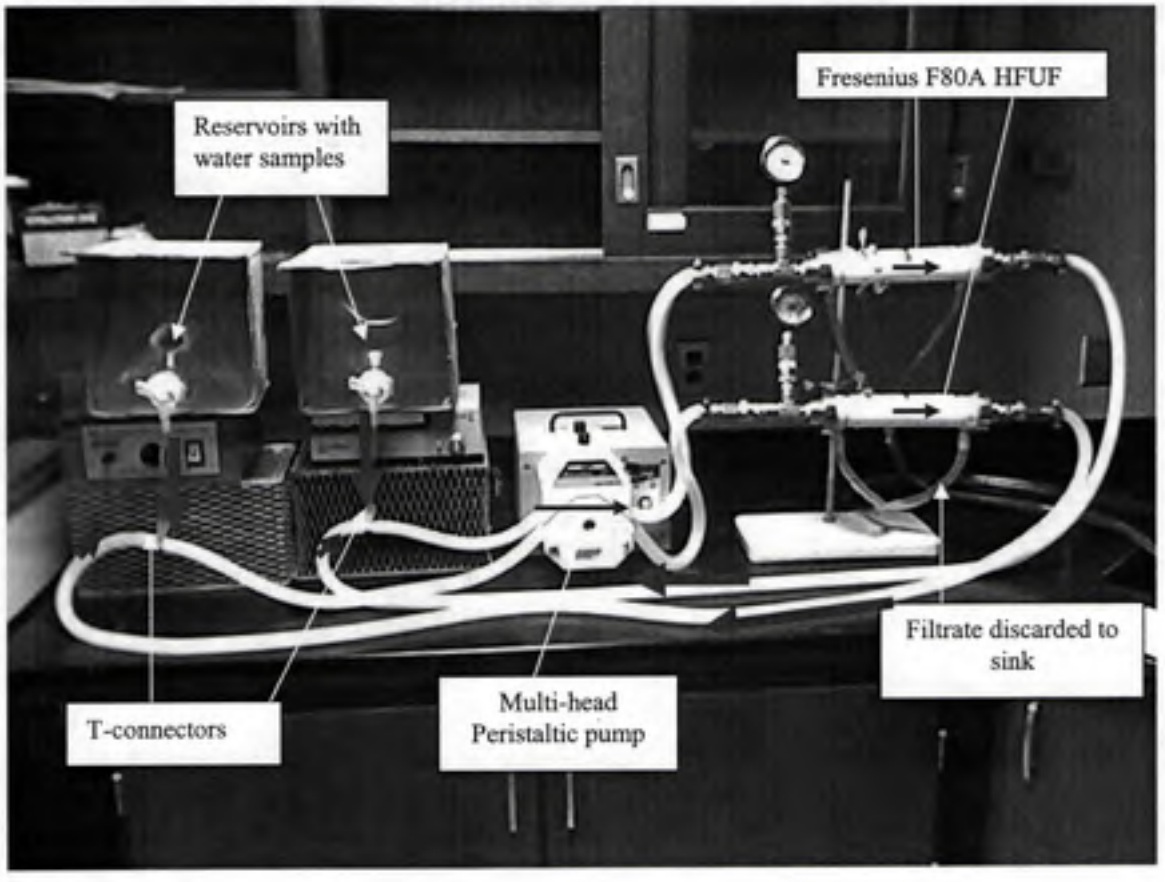


2. Modification of HFUF Endcaps for Increased Flow

Unpublished reports have demonstrated the utility of HFUFs yielding higher recovery efficiencies and shorter sample processing times through the use of physical modifications to the ultrafilter housing inlet and outlet openings. These modifications (Figure 7) primarily affect the flux of the substrate through the filter by increasing the cross-sectional surface area that the filter is exposed to the fluid. An increase in the flux (Q), with no other changes in Darcy's Law (Figure 1) must cause a decrease in processing time to remain in a steady state. These modifications were tested by modification and replacement of the original end caps with ones constructed of stainless steel and were produced by the UNC Department of Physics using a CNC (computer aided) milling machine (Figure 8). The modified end caps use a double-helical metric thread (similar to a large Leur thread) that mates with the existing threads on the ends of the HFUF. The new end caps were also modified to have a female ½"

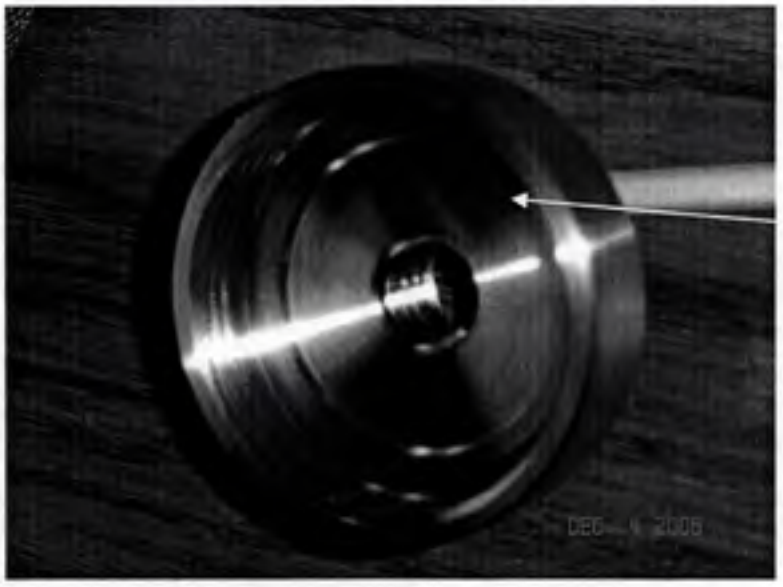
NPT thread for which to plumb appropriate hardware for connection to the system. Masterflex tubing was used with ½" internal diameter along with a larger appropriate peristaltic pump (Burt Process Equipment, Inc. Hamden, CT Masterflex I/P Precision Brushless Drive) capable of achieving a recirculatory flow rate through the HFUF system of 13 liters per minute. The system was operated at a maximum of 25 psi.

Figure 7. Modified HFUF setup (processing multiple samples)



* Flow pattern marked by heavier arrows

Figure 8. Alternative Endcaps Tested



Microbial recovery trials were conducted with 10L water volumes seeded with indicator microorganisms, as previously outlined. Samples of the initial water, sample concentrate, as well as sample filtrate, were serially diluted and assayed, to assess method recovery efficiency. Studies compared conventional and modified HFUF end caps and results were statistically compared for microbial recoveries and sample processing times using both source and treated waters. Flow rates were recorded for the time, in minutes, required to process the entire 10L cubitainer then converted to L/min for flow rates for standardization.

3. Alternative Eluting Solutions for Recovery of Concentrated Microbial Indicators

An eluting solution is passed through the HFUF system following initial concentration to promote non-specific binding of particles within the filter and tubing allowing for recovery of microorganisms by the system. Three eluting solutions tested in

these experiments had relatively similar properties, but varying slightly in chemical compositions with the surfactant and antifoam solution being the only constant additives due to their importance in the eluting process. PBS continued to prove to be an important additive that has been seen in previous literature due to its prevention of microbial attachment leading to the promotion of recovery.(Simmons, Sobsey et al. 2001; Morales-Morales, Vidal et al. 2003) The eluting solutions generally consisted of a buffered solution supplemented with a non-ionic detergent which in these cases was the Tween 80. These eluting solutions were chosen due to their previous use (Standard), their described use in the literature (Solution 2), or their use for recovery of viruses from charge-modified electro-positive filters (Cuno Virosorb 1MDS filters) (Solution 3) (Simmons, Sobsey et al. 2001; Morales-Morales, Vidal et al. 2003). Each of these were tested with both the modified and conventional HFUF setups and statistically compared to determine the most efficient eluting solution for microbe recovery. Eluting solution 1 is the standard eluting solution, used in other experiments and was to be the baseline eluting solution for the comparison of the modified types. This eluting solution was used in prior experiments for the recovery of *Cryptosporidium parvum* (Simmons, Sobsey et al. 2001) and relies on using a buffered solution, PBS, to prevent damage to the microorganisms for more reliable results, Elution solution 2 used the addition of .the sodium polyphosphate (NaPP), which was used in previous experiments as well, but not as an eluting solution additive, rather a water sample additive (Hill, Polaczyk et al. 2005). Eluting solution 3 was also used in previous literature, but not for the elution of multiple classes of microbes from hollow-fiber ultrafilters. The three test eluting solutions were as follows:

Eluting Solution 1 (Standard)

1 L Phosphate-buffered Saline (PBS)

10 g laureth-12

50 µL antifoam-A

Eluting Solution 2

1 L Phosphate-buffered Saline (PBS)

10 g laureth-12

1 g Sodium polyphosphate (NaPP)

50 µL antifoam-A

Eluting Solution 3

1 L reagent water

52.675 g L-Arginine (A-5131) (0.25 M final concentration)

45.65 g L-Lysine (L-5826) (0.25 M final concentration)

10 g laureth-12

50 µL antifoam-A

4. Use of Multiple HFUFs for Concentrating Microbial Indicators in Water

In order to increase the efficiency for the HFUF system, further hydraulic modifications were tested by using multiple HFUFs in parallel (Figure 9) and in series (Figure 10). These modifications were based on proven hydraulic design flow paths. These hydraulic modifications were tested with both the conventional setup as well as with the

modified end caps to determine microbe recovery, flow capacity, and sample processing times. This use of more than one HFUF to process a sample primarily affects the surface area for hollow-fiber ultra filtration. An increase in Surface Area (A) with no other changes in Darcy's Law (Figure 1) will cause an increase in Flux or Volumetric Flowrate (Q) of the water through the filter which should decrease processing time to remain in steady state. The use of multiple HFUFs without the modified end caps would also allow laboratories to increase hollow-fiber ultra filtration system efficiency without the costs associated with design and manufacture of the modified end caps, larger peristaltic pump and associated hardware for using the modified end caps.

Figure 9. Parallel Flow HFUF Hydraulic Design with Modified Endcaps

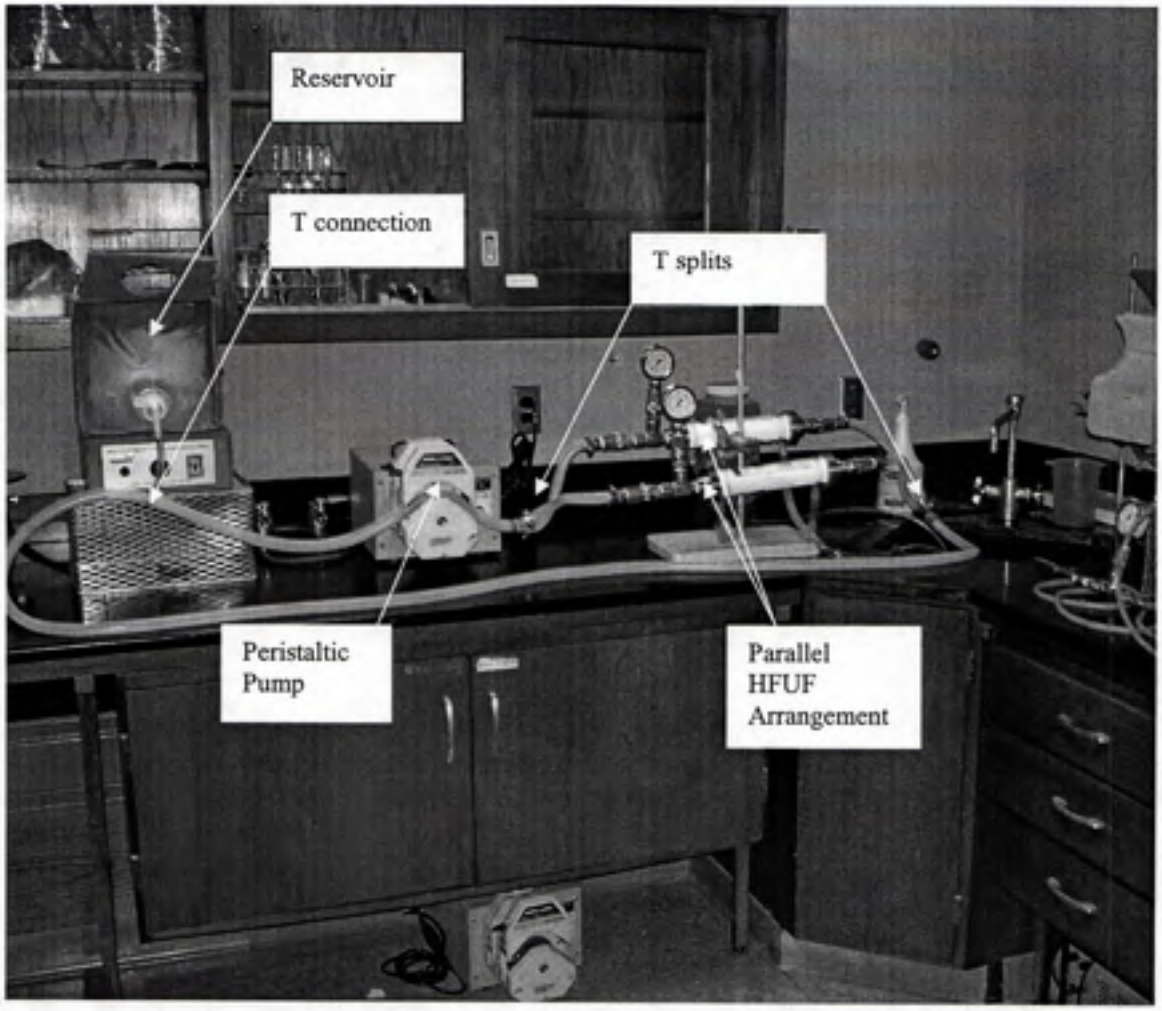
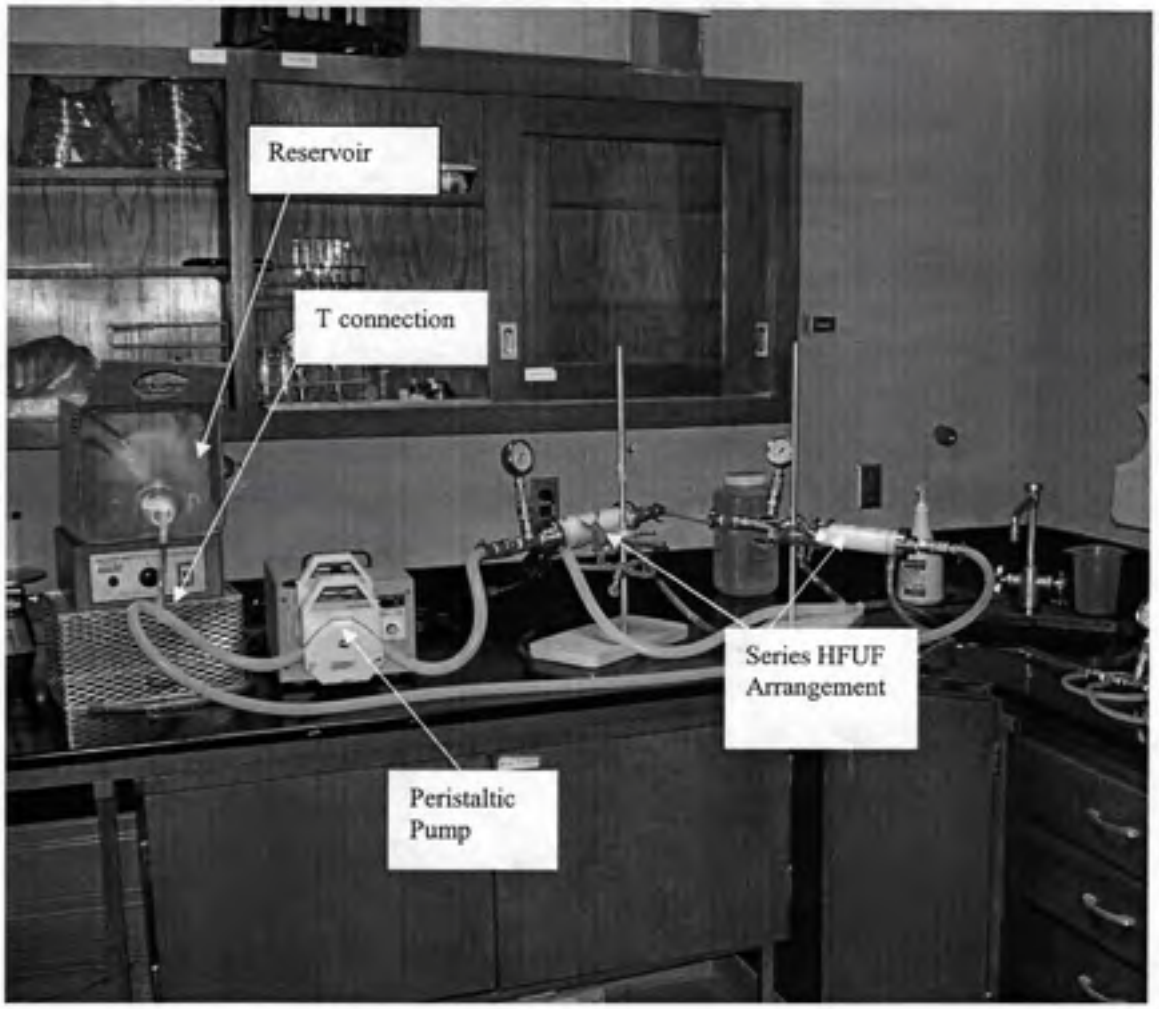


Figure 10. Series Flow HFUF Hydraulic Design with Modified Endcaps



D. Statistical Analysis of Data

Paired, non-parametric statistical analyses were used to compare filter recoveries from water samples using a computer-based statistical software package (Instat, GraphPad Software, San Diego, CA). Specifically, for experiments to compare two sets of data, a Mann-Whitney analysis was used to compare water parameters (SFPUC verses OWASA) filter design (F80A verses F200A), filter modification (modified end caps verses

conventional end caps) and hydraulic design modifications (series verses parallel) on recovery rate of microorganisms as well as flow rates for each HFUF trial. For experiments that compared more than two categories of data, a Kruskal-Wallace non-parametric analysis of variance (ANOVA) or Friedman non-parametric repeated measured analysis of variance (ANOVA) was used.

V. RESULTS

A. Physical and Chemical Parameters of Test Waters

Physical and chemical parameters of test waters may influence efficiency as well as processing time for microbial recovery methods. For these experimental trials, test waters were either source or treated drinking waters and multiple microbial recovery trials were performed with each water type. Ideally, each water type would have similar physical and chemical characteristics so that recovery efficiencies and processing times could be directly attributable to test conditions, but because the waters were collected from different geographic locations and the treated water samples were processed by different utilities using different processing techniques, the waters proved to be dissimilar for the physical and chemical parameters measured. To ensure reliability and robustness for the method, the waters were analyzed separately for statistical differences in method recovery efficiency and for sample processing times. Parameters measured for both types of water samples included pH, conductivity, alkalinity, hardness, chlorine residual (treated only), and total coliform bacteria counts (treated only). These physical and chemical parameters were measured by the water utility from which the test waters were collected and results were forwarded to the UNC Microbiology Laboratory to be analyzed for correlations with key performance results of these experiments.

Source water was obtained from 11/9/2006 to 3/2/2008 and consisted of waters drawn from two independent sources, the Hetch Hetchy Reservoir at Yosemite National Park (Table

1) and Cane Creek Reservoir in Orange County North Carolina (Table 2). The physical parameters for each of the water samples within their subsequent reservoirs were generally consistent over the duration of the project showing no significantly statistical deviations for all parameters measured. A Friedman non-parametric repeated measures ANOVA test was performed with no statistically significant differences observed for any of the test waters collected from each water utility (Friedman Test; $p=0.3314$ and 0.2797 for SFPUC and OWASA respectfully) with larger statistically significant deviations seen between reservoirs as would be expected. Water pH ranged from 9.0 to 9.4 in the OWASA waters and 6.7 to 6.9 in the Hetch Hetchy waters. When compared, they showed significant statistical differences (Mann-Whitney Test; $p=0.0022$). The Hetch Hetchy water showed lower turbidities (0.3 to 0.4 NTU) than in the Cane Creek Reservoir (2.0 to 6.9 NTU), which were also statistically different (Mann-Whitney Test; $p=0.0022$). Hardness and alkalinity remained similar in the Hetch Hetchy waters at 6 mg/L while the Cane Creek water showed a larger variance with hardness ranging from 28 to 42 mg/L and alkalinity ranging from 24 to 42mg/L. Upon comparison, both parameters showed significant differences between water sources (Paired T test; $p=<0.0001$, 0.0005 respectively). Total Organic Content (TOC) was also compared for the two reservoirs, with the Cane Creek waters showing higher levels as well as more variability (6.0-6.8mg/L) than the Hetch Hetchy waters (0.9-1.1mg/L). These data showed continued to show the significant statistical differences (Mann-Whitney Test; $p=0.0022$) between the waters. Therefore, the physical and chemical parameters for the water samples were similar amongst the reservoirs, but not between the two reservoirs and were separated for all further analyses to ensure no pronounced effects on observed differences in the recovery efficiency for the experimental trials using source waters

Table 1. Physical Parameters of San Francisco Source Water

Sample collection date**	pH	Turbidly (NTU)	Hardness (mg/L)	Alkalinity (mg/L)	TOC* (mg/L)
11/9/2006	9.0	0.3	6	6	1.0
11/28/2006	9.4	0.4	6	6	1.0
1/17/2007	9.2	0.4	6	6	1.0
1/23/2007	9.0	0.3	6	6	0.9
1/31/2007	9.1	0.3	6	6	1.1
3/22/2007	9.3	0.3	6	6	1.0

*TOC Total Organic Content mg/L

Table 2. Physical Parameters of Orange County Source Water

Sample collection date**	pH	Turbidly (NTU)	Hardness (mg/L)	Alkalinity (mg/L)	TOC* (mg/L)
9/10/2007	6.7	2.0	28	24	6.8
1/29/2008	6.9	5.7	29	42	6.5
2/4/2008	6.9	6.5	30	40	6.0
2/15/2008	6.8	6.9	30	26	6.4
2/20/2008	6.9	5.7	29	26	6.4
3/2/2008	6.9	5.7	42	29	6.5

*TOC Total Organic Content mg/L

Treated water was obtained from 10/30/2006 to 1/23/2008 with a total of 16 samples received from SFPUC (Table 3) and OWASA (Table 4). Treated water samples from SFPUC were de-chlorinated with sodium thiosulfate (2 mg/L) prior to shipping to the UNC-CH laboratories and OWASA water samples were de-chlorinated within 2 hours prior to processing. Most water quality parameters remained constant among water sources with the highest variability noted in hardness (ranging from 12 to 62 mg/L) for SFPUC waters and alkalinity (ranging from 37 to 47 mg/L) for OWASA waters. Both SFPUC and OWASA waters were also screened for the presence of total coliform bacteria with no positive results

over the duration of these trials. It should also be noted that the experimental schedule was adjusted during the time that the water usage from the Hetch Hetchy reservoir was suspended in late February through March, 2007 in order to have consistency throughout experimental trials. Similar statistical comparisons to that of the source waters were made using a Friedman non-parametric repeated measures ANOVA with no statistically significant differences observed for any of the test waters collected from each water utility (Friedman Test, p=0.4809, 0.4294 for SFPUC and OWASA water respectfully).

Upon comparison of the treated waters from SFPUC and OWASA, statistically significant differences were found for most parameters tested. The pH was less variable and higher, ranging from 8.8 to 9.1 in SFPUC waters when compared to OWASA waters where pHs varied from 7.8 to 9.2, which showed statistically significant differences (Mann-Whitney Test; p=0.0002). Total chlorides varied from 1.3 to 1.8 mg/L in the SFPUC waters which was lower overall than the 3.1 to 3.4 mg/L found in the OWASA waters, which showed a statistically significant difference between the two water utilities (Mann-Whitney Test; p=0.00002). Turbidity was higher in the SFPUC waters as well as more variable than in the OWASA waters ranging from 0.1 to 0.3 NTU while OWASA waters showed a steady level of 0.1 NTU, also differing significantly between water utilities (Mann-Whitney Test; p=0.0045) Chlorides were more variable in the SFPUC waters (3 to 15mg/L) than in the OWASA waters (3 to 4 mg/L), and showed no statistical differences when compared (Mann-Whitney Test; p=0.7480). Hardness and alkalinity were compared among the water facilities, with hardness showing no statistical significance between the two (Mann-Whitney Test; p=0.0712) and alkalinity showing statistically significance differences between the two waters (Mann-Whitney Test; p=0.0164).Therefore, the physical water parameters for the

treated waters were similar among individual water utilities resulting in no pronounced differences observed in the recovery efficiency accountable to unknown matrix effects associated with these waters for the experimental trials resulting in separation of waters by the individual utilities as well as by water type.

Table 3. Physical Parameters of San Francisco Treated Water

Sample collection date**	pH	Total Cl2 (mg/L)	Turbidly (NTU)	Total Coliform (P/A)*	Cl - (mg/L)	Hardness (mg/L)	Alkalinity (mg/L)
10/30/2006	9.2	1.8	0.3	A	3	12	12
11/6/2006	9.1	1.7	0.3	A	3	12	12
12/7/2006	9.2	1.6	0.4	A	3	14	14
12/13/2006	9.1	1.7	0.4	A	3	14	14
2/8/2007	8.8	1.4	0.2	A	6	28	28
2/15/2007	8.9	1.3	0.2	A	8	32	32
2/22/2007	8.8	1.8	0.1	A	15	62	58

*Total Coliform (P/A) – presence or absence of total coliform bacteria in waters as measured by SFPUC WQB and OWASA Laboratories

Table 4. Physical Parameters of Orange County Treated Water

Sample collection date**	pH	Total Cl2 (mg/L)	Turbidly (NTU)	Total Coliform (P/A)*	Cl - (mg/L)	Hardness (mg/L)	Alkalinity (mg/L)
8/27/2007	8.1	3.4	0.1	A	4	30	37
9/17/2007	8.1	3.2	0.1	A	4	30	45
10/1/2007	8.0	3.2	0.1	A	3	30	47
10/15/2007	8.0	3.3	0.1	A	4	30	47
11/15/2007	8.2	3.5	0.1	A	4	30	46
12/17/2007	7.8	3.1	0.1	A	3	33	40
1/7/2008	8.2	3.3	0.1	A	4	35	37
1/14/2008	8.1	3.1	0.1	A	3	37	42
1/23/2008	7.8	3.1	0.1	A	3	35	40

*Total Coliform (P/A) – presence or absence of total coliform bacteria in waters as measured by SFPUC WQB and OWASA Laboratories

B. Recovery of Microbial Indicators using Modified HFUF Endcaps

In order to overcome lengthy processing times, modifications were made to the system for enhanced flow rates through the HFUF. Modified endcaps (Figure 8) had larger diameter openings, tubing had a larger inside diameter, and a more powerful pump, was used, in an attempt to increase sample flow and decrease sample processing time while maintaining method recovery efficiency. Two groups of experiments were performed based on water type (source and treated drinking waters) for each water utility. For each water type, two sample processing designs were compared in parallel: the conventional design (Figure 5) and the modified higher flux design (Figure 9). The Fresenius F80A HFUF was used for the source waters, and the Fresenius F200A was used for the treated waters.

Table 5 shows the microbial recoveries of individual trials as well as average recovery and standard deviations for the microbial indicator recoveries by the Fresenius F80A HFUFs in source waters from OWASA. *E. coli* KO11 showed a higher recovery rate of 61±13% using the modified design when compared with 57±13% achieved using the conventional design. The coliphage MS-2 and *Bacillus atrophaeus* recovery rates of 74±13% and 61±12% with the modified endcaps were lower than those of the conventional endcaps which were 84±17% and 67±11% respectively. When microbe recovery rates were statistically compared using a Mann-Whitney non-parametric analysis there were no statistically significant differences for any of the microbe recovery efficiencies observed (Mann-Whitney Test of *E. coli*, coliphage MS-2, and *Bacillus atrophaeus*; p values of 0.6723, 0.5251, and 0.2409 respectively). This demonstrates that the endcap design types (conventional and modified endcaps) are equivalent for microbial recovery efficiency in concentrating these microbial indicators from source waters.

Average filtrate rate of the conventional setup was 0.59±0.06 L/min while the modified setup was 0.65±0.16 L/min (Table 5). This indicated a 0.06 L/min increase in filtrate flow using the modified system when compared with the conventional endcap assembly provided with the HFUF. When filtrate rates were statistically compared using a Mann-Whitney Test there was no statistically significant differences in rates when conventional or modified endcaps were used (Mann-Whitney Test; p value = 0.8539). This demonstrates that HFUF endcap design types (conventional and modified endcaps) yield similar filtrate flow rates for the source waters tested.

Table 5. Concentration and Recovery of Indicator Organisms in Orange County Source Waters by Conventional and Modified Endcaps (Fresenius F80A)

Organism	Conventional			Modified		
	Flowrate (L/min)	Trials (N)	Average Recovery (%)	Flowrate (L/min)	Trials (N)	Average Recovery (%)
E. coli K011	0.59±0.06	5	181±56	0.65±0.16	4	72±40
Coliphage MS-2		5	78±21		5	92±22
Bacillus atrophaeus		5	135±49		4	127±54
No Significant Difference by Mann Whitney Test for *E. coli*, coliphage MS-2, and *Bacillus atrophaeus*; p values of 0.2857, 0.5476, and 0.9048, respectively.						

Table 6 shows microbial recoveries of individual trials as well as average recoveries and standard deviations for the microbial indicator recoveries by the Fresenius F80A HFUFs in treated drinking waters from SFPUC. All three indicator organisms showed lower recovery rates using the modified endcaps with average recovery rates of 60±21%, 85±12%, and 57±13% for the modified endcaps as compared to 112±36%, 109±18%, and 71±19% using the conventional system for *E. coli*, coliphage MS-2, and *Bacillus atrophaeus* respectfully. A

Mann-Whitney analysis showed no statistically significant differences for any of the microbe recovery percentages tested (Mann Whitney Test of *E. coli*, coliphage MS-2, and *Bacillus atrophaeus*; p values = 0.0874, 0.5789, and 0.5663, respectively). Therefore, the endcap design types (conventional and modified endcaps) are equivalent for microbial recovery efficiencies from treated drinking waters.

Filtration rates for treated drinking waters were 0.17±0.02 L/min for the conventional setup and 0.46±0.04 L/min for the modified system (Table 6). This equates to a 0.29 L/min increase in filtrate rate with the modified setup, which is significantly higher than that with the conventional system (Mann Whitney Test; p value <.0001). Therefore, the modified endcap design significantly increases the filtrate rate through the filter with treated drinking water thereby effectively reducing sample processing times by 2.7-fold.

Table 6. Concentration and Recovery of Indicator Organisms in Orange County Treated Drinking Waters by Conventional and Modified Endcaps (Fresenius F200A)

Organism	Conventional			Modified		
	Flowrate (L/min)	Trials (N)	Average Recovery (%)	Flowrate (L/min)	Trials (N)	Average Recovery (%)
E. coli K011	0.17±0.02	6	112±36	0.46±0.04	13	60±21
Coliphage MS-2		6	109±18		13	85±12
Bacillus atrophaeus		5	71±19		13	57±13
No Significant Difference by Mann Whitney Test for *E. coli*, coliphage MS-2 and *Bacillus atrophaeus*; p values of 0.0874, 0.5789, and 0.5663, respectively.						

C. Recovery of HFUF Concentrated Microbial Indicators by Multiple Eluting Solutions

Following microbial concentration by hollow-fiber ultrafiltration, an eluting solution is used to assure recovery of concentrated microbial pathogens and indicators in the HFUF

retentate. Three eluting solutions were tested for efficiency in recovering HFUF-concentrated microbial indicators from source waters. Eluting solutions 1 and 2 have been previously used for HFUF recovery and Eluting solution 3 was chosen based on its use for recovering viruses from Cuno Virosorb 1MDS charge modified filters (Sobsey and Jones 1979). Average microbial recoveries by eluting solutions ranged from a low of 49±24% for *E. coli* by solution 2 to 132±32% for *E. coli* by solution 1. Eluting solution 1 gave highest recoveries for two of the three classes of indicator organisms (*E. coli* KO11, *Bacillus atrophaeus*) (Table 7). When the three eluting solutions were statistically compared using a Kruskal-Wallis Nonparametric ANOVA, average recovery efficiencies were not statistically significant different with *E. coli*, coliphage MS-2, and *Bacillus atrophaeus* p values of 0.3653, 0.3535, and .3854, respectively. These results demonstrate no differences in recovery efficiencies by the three eluting solutions tested for microbial indicators concentrated from source waters.

Table 7. Average HFUF Recovery of *E. coli*, Coliphage MS-2, and *Bacillus atrophaeus* in San Francisco Source Waters Using Multiple Eluting Solutions

Organism	Eluting Solution 1		Eluting Solution 2		Eluting Solution 3	
	Trials (N)	Average Recovery (%)	Trials (N)	Average Recovery (%)	Trials (N)	Average Recovery (%)
E. coli K011	16	132±32	3	49±24	1	51
Coliphage MS2	15	77±11	3	103±27	3	122±41
Bacillus atrophaeus	15	131±24	3	63±22	3	74±29
No Significant Differences by Kruskal-Wallis ANOVA for *E. coli*, coliphage MS-2 and *Bacillus atrophaeus*; p values = 0.3653, 0.3535, and 0.3854, respectively.						

*All experiments performed with modified endcaps on F80A HFUFs

The three eluting solutions were also tested for recovery of concentrated microbial indicators from treated drinking water. Average microbial recoveries by eluting solutions

ranged from a low of 24±16% for *Bacillus atrophaeus* by solution 3 to a high of 89±09% for MS-2 by solution 1. Similar to trials with source waters, recovery efficiencies were higher with eluting solution 1 for two of the three classes of organisms (*E. coli* KO11, coliphage MS-2) than eluting solution 2 or 3 (Table 8). When the three eluting solutions were statistically compared using a Kruskal-Wallis Nonparametric ANOVA, average recovery efficiencies were not statistically significant different with *E. coli*, coliphage MS-2, and *Bacillus atrophaeus* with p values of 0.3653, 0.3535, and .3854, respectively. As with the trials in source waters, these results demonstrate no differences in microbial recovery efficiencies by the three eluting solutions tested.

Table 8. Average HFUF Recovery of *E. coli*, Coliphage MS-2, and *Bacillus atrophaeus* in San Francisco Treated Drinking Waters Using Multiple Eluting Solutions

Organism	Eluting Solution 1		Eluting Solution 2		Eluting Solution 3	
	Trials (N)	Average Recovery (%)	Trials (N)	Average Recovery (%)	Trials (N)	Average Recovery (%)
E. coli K011	36	71±12	6	50±07	3	57±15
Coliphage MS2	35	89±09	6	54±04	3	82±10
Bacillus atrophaeus	35	63±6	6	78±33	3	24±16
No Significant Differences by Kruskal-Wallis ANOVA for *E. coli*, coliphage MS-2 and *Bacillus atrophaeus*; p values = 0.8757, 0.1920, and 0.1666 respectively.						

*All experiments performed with modified endcaps on F80A HFUFs

D. Microbial Indicator Recovery by HFUFs having Different Design Patterns

Two HFUFs were tested in these trials to determine the effects of varied tubule designs on microbe recovery, the achievable flux by the HFUF system, and corresponding changes in sample processing times. The two HFUF models tested were manufactured by Fresenius Medical Care AG & Co. KGaA ,Bad Homburg, Germany and were the Hemoflow™ F80A (parallel tubing) and the Fresenius Optiflux® F200A (higher surface area,

more turgid flow pattern). Other than the tubular designs within the HFUF, these had similar properties; the largest difference between them was price (F80A approximately $40; F200A more than $100).

The Fresenius Hemoflow™ F80A and Fresenius Optiflux® F200A were tested in source waters from OWASA with the modified endcaps to determine recovery efficiency for *E. coli*, coliphage MS-2, and *Bacillus atrophaeus* (Table 9). The F80A had the higher recovery rate for two of the three microbes tested (*E. coli* KO11, coliphage MS-2). *E. coli* KO11 had a recovery rate of 133±39% (F80A) as compared to 95±10% (F200A) and coliphage MS-2 had a recovery rate of 85±14% (F80A) as compared to 78±25% (F200A). *Bacillus atrophaeus* showed a lower recovery rate of 188±64% for the F80A as compared to 211±18% in the F200A. Microbial indicator recoveries achieved by these two HFUFs in source waters showed no statistically significant differences (Mann Whitney Test of *E. coli*, coliphage MS-2, and *Bacillus atrophaeus*; p values = 0.7273, >0.9999, and 0.3607, respectively). Therefore, filter tubule design had no pronounced effects on microbial indicator recovery efficiency for these experimental trials in source waters.

Table 9. Concentration and Recovery of Indicator Organisms in Orange County Source Waters using Fresenius Hemoflow™ F80A and Optiflux® F200A

Organism	F80A		F200A	
	Trials (N)	Average Recovery (%)	Trials (N)	Average Recovery (%)
E. coli K011	9	133±39	3	95±10
Coliphage MS-2	10	85±14	3	78±25
Bacillus atrophaeus	9	188±64	3	211±18
No Significant Difference by Mann Whitney Test of *E. coli*, coliphage MS-2, and *Bacillus atrophaeus*; p values = 0.7273, >0.9999, and 0.3706, respectively.				

*All experiments performed with modified endcaps of both filter types

Filtrate rates were not compared between the F80A and the F200A for source waters during these experimental trials (Table 10). The Fresenius F80A HFUF with the conventional endcaps had an average filtrate rate of 0.65±0.16 L/min. No trials were performed for the F200A using the conventional endcaps provided by the manufacturer. Because of this, flow type cannot be accounted for in this conventional setup. Recovery rate comparisons of microbial organisms were conducted using the F200A and the F80A in treated waters, however, they could not be compared due to a difference in water characteristics found in the OWASA and SFPUC treated water physical and chemical parameters.

Table 10 Filtration Rates for Fresenius F80A Ultrafilters in OWASA Source Waters with Different Hydraulic Configurations: Single Filters and Dual Filters in Parallel or in Series

	Single		Parallel		Series	
	Trials (N)	Filtrate Rate (L/min)	Trials (N)	Filtrate Rate (L/min)	Trials (N)	Filtrate Rate (L/min)
Conventional	5	0.65±0.16	2	0.46±0.13	3	1.75±0.25
Modified	10	0.59±0.06	2	0.59±0.24	3	1.81±0.19
Significant Differences by Kruskal-Wallis Test (Nonparametric ANOVA) for conventional and modified hydraulic modifications; p values = 0.0639 and 0.0323 respectfully under a reduced level of significance ($p<0.1$)						

E. Microbial Indicator Recovery Using Multiple HFUFs in Parallel and Series

Previous experiments focused on increasing the water filtration rate of HFUFs while maintaining microbial indicator recovery efficiency by increasing flux using modified endcaps and by use of alternative HFUFs (Fresenius F80A versus F200A). In an attempt to further increase flux and thereby reduce sample processing times, the HFUF surface area was increased by using two HFUFs instead of a single HFUF. All previous experimental trials in the literature used only a single filter. The hollow-fiber ultrafiltration systems were plumbed with the two HFUFs either in series or in parallel. Each of these modifications in filter setup

were tested in source and treated drinking waters for microbial recovery efficiency, while also noting sample filtration rates and the goal of reduced sample processing times.

1. Recovery of Microbial Indicators from Untreated Source Waters by Multiple HFUFs

The use of two HFUFs in parallel was tested for microbial recovery efficiency and sample processing time in OWASA source water in comparison with only a single HFUF (Table 11). Comparing the single and two in parallel HFUFs, average recoveries were 123±29% and 99±43% respectively for *E. coli* K011, 83±12% and 60±10%, respectively, coliphage MS-2, and 151±27% and 165±133% respectively for *Bacillus atrophaeus*. When recovery efficiencies for the single and two in parallel HFUFs were statistically compared, there were no significant differences for *E. coli*, coliphage MS-2, and *Bacillus atrophaeus* by Mann Whitney Test (p=>0.9999, 0.5487, and 0.6649, respectively.). These results demonstrate that use of two HFUFs in parallel versus using only a single HFUF did not change microbial recovery efficiency from source waters.

Table 11. Concentration and Recovery of Indicator Organisms in OWASA Treated Drinking Waters using Fresenius Hemoflow™ F80A with Conventional Endcaps

	Single		Parallel	
Organism	Trials (N)	Average Recovery (%)	Trials (N)	Average Recovery (%)
E. coli K011	12	123±29	4	99±43
Coliphage MS-2	13	83±12	4	60±10
Bacillus atrophaeus	12	151±27	3	165±133
No Significant Difference in recovery of *E. coli*, coliphage MS-2, and *Bacillus atrophaeus* by single and dual parallel HFUFs, with Mann-Whitney p values of >0.9999, 0.5487, and 0.6649, respectively.				

A Fresenius F80A with conventional endcaps was used as a basis of comparison in trials on the performance of two HFUFs either in parallel or in series. Average filtration rates were 0.65±0.16 L/min for the single HFUF setup and 0.46±0.13 L/min for two HFUFs in parallel. This filtration rate difference of 0.19 L/min. when using two HFUFs in parallel compared to a single HFUF was not statistically significant (Mann Whitney Test; $p = 0.6973$). The Fresenius F80A HFUF with the modified endcap system was the basis for comparison of microbial recoveries from source waters when using two HFUFs either in parallel or in series. Filtration rates were 0.59±0.06 L/min and 0.59±0.24 for the single HFUF and two HFUFs in parallel, and they were not statically significantly different (Mann Whitney Test; $p = 0.9143$) Two HFUFs in series were also tested in comparison with a single HFUF for microbial recoveries (Table 12). Average microbes recoveries of single HFUF versus two HFUFs in series were 123±29% versus 58±32% for *E. coli* K011, 83±12% versus 106±30% for coliphage MS-2, and 151±27% versus 103±27% for *Bacillus atrophaeus*. For each test microbe recovery efficiencies by single HFUFs and two HFUFs in series were not significantly different (Mann Whitney Test p values of 0.1797, 0.7012, and 0.2496, respectively

Table 12. Microbial Indicator Recovery using Single and Multiple HFUF in Parallel from OWASA Source Waters

	Single		Series	
Organism	Trials (N)	Average Recovery (%)	Trials (N)	Average Recovery (%)
E. coli K011	12	123±29	6	58±32
Coliphage MS-2	13	83±12	6	106±30
Bacillus atrophaeus	12	151±27	6	103±27
No Significant Difference by Mann Whitney Test for *E. coli*, coliphage MS-2, and *Bacillus atrophaeus*; p values of 0.1797, 0.7012, and 0.2496, respectively.				

*All experiments performed with modified endcaps on F80A HFUFs

Two HFUFs with conventional endcaps and placed in series were compared with a single, Fresenius F80A with conventional endcaps for concentrating microbial indicators from source waters and was the basis of comparison to the system using two HFUFs in series. Average flow rates were of 0.65±0.16 L/min and 1.75±0.25 L/min, respectively, for the single HFUF with conventional endcaps and the two HFUFs with conventional endcaps in series. The average filtrate rate difference of 1.10 L/min. for the two HFUF systems was not quite statistically significantly different (Mann-Whitney Test; p value = 0.0520). The system of two HFUFs with conventional endcaps in series may have a greater filtration rate and faster sample processing time than a single unit. Two HFUFs with modified endcaps and placed in series were compared with a single Fresenius F80A with modified endcaps for concentrating microbial indicators from source waters. Average flow rates were of 0.59±0.06 L/min and 1.81±0.19 L/min, for the single HFUF with modified endcaps and two HFUFs with modified endcaps in series. The average filtrate rate difference of 1.22 L/min. for the two HFUF systems was statistically significantly different (Mann-Whitney Test; p value = 0.007). The system of two HFUFs with conventional endcaps in series has a higher filtration

rate than a single unit, which indicates a shorter sample processing time over the system using only a single HFUF.

2. Recovery of Microbial Indicators by Multiple HFUFs from Treated Drinking Waters

The use two HFUFs in parallel was tested for microbial recovery efficiency and for sample processing times in treated drinking water and compared to a use of a single HFUF (Table 13). Average recoveries for a single HFUF versus two HFUFs in parallel were 77±18% versus 79±13% , respectively, for *E. coli* K011, 92±10% versus 97±10%, respectively for coliphage MS-2, and 61±11% versus 71±10%, respectively, for *Bacillus atrophaeus* Recoveries of each test microbe (*E. coli*, MS-2 and *Bacillus atrophaeus*) by the two HFUF systems (single versus two in parallel) were not statistically significantly different by Mann-Whitney Test, with p values of 0.2736, 0.9260, and 0.3432, respectively. Therefore, using two HFUFs in parallel or a single HFUF yielded equivalent test microbe recovery efficiencies from treated drinking waters.

Table 13. Microbial Indicator Recovery using Single and Multiple HFUF in Series from OWASA Source Waters

	Single		Parallel	
Organism	Trials (N)	Average Recovery (%)	Trials (N)	Average Recovery (%)
E. coli K011	19	77±18	6	79±13
Coliphage MS2	19	92±10	6	97±10
Bacillus atrophaeus	18	61±11	6	71±10
Data combined for comparison of median recoveries of indicator organisms. No Significant Difference by Mann Whitney Test of *E. coli*, Coliphage MS-2, and *Bacillus atrophaeus* with p values of 0.2736, 0.9260, and 0.3432 respectively.				

*All experiments performed with modified endcaps on F80A HFUFs

A single Fresenius F200A with conventional endcaps was compared to a system using two HFUFs in parallel (Table 14) based on filtrate rates of treated water. Average treated water filtrate rates of a single HFUF versus two HFUFs in parallel were 0.57±0.14 L/min, versus 0.79±0.10 L/min, and the average filtration rate difference of 0.22 L/min. was not statistically significant (Mann-Whitney Test p value = 0.3810). Therefore, treated drinking water filtration rates for systems using a single HFUF or two HFUFs in parallel were equivalent. A Fresenius F200A with modified endcaps was compared to the system using two HFUFs in parallel, based on filtration rates. Average treated water flow rates of HFUFs with modified endcaps tested singly versus two in parallel were 0.66±0.03 L/min, versus 1.15±0.04 L/min, and the average filtration rate difference of 0.49 L/min. was statistically significant (Mann-Whitney Test p value = 0.0009). Therefore, treated drinking water filtration rates for systems using HFUFs with modified endcaps either singly as two in parallel were not equivalent, with greater flow rates and shorter processing times using the latter system.

Table 14. Filtrate Rates of Fresenius F200A Ultrafilters in OWASA Treated Waters with Different Hydraulic Modifications to the System

	Single		Parallel		Series	
	Trials (N)	Filtrate Rate (L/min)	Trials (N)	Filtrate Rate (L/min)	Trials (N)	Filtrate Rate (L/min)
Conventional	6	0.57±0.14	3	0.79±0.10	3	1.25±0.24
Modified	22	0.66±0.03	3	1.16±0.05	3	1.62±0.19
Significant Differences by One way ANOVA for conventional and modified hydraulic modifications; p values = 0.0489,and <0.0001 respectively						

Similar experimental trials tested microbial recoveries from treated drinking water using a single HFUF and two HFUFs in series. Average microbe recoveries by HFUFs used single versus two in series were 77±18% versus 114±75% , respectively, for *E. coli* K011, 92±10% versus 71±25%, respectively, for coliphage MS-2, and 61±11% versus 55±12%, respectively, for *Bacillus atrophaeus* (Table 15). These average microbial indicator recoveries of *E. coli*, coliphage MS-2, and *Bacillus atrophaeus* were not statistically significant by Mann Whitney Test, with p values of 0.7846, 0.3657, and 0.8375, respectively). These results indicate equivalent microbial recovery efficiency from treated water by HFUFs used either singly as two in series.

Table 15. Microbial Indicator Recovery using Single and Multiple HFUF in Parallel from in Orange County Treated Drinking Waters

	Single		Series	
Organism	Trials (N)	Average Recovery (%)	Trials (N)	Average Recovery (%)
E. coli K011	19	77±18	4	114±75
Coliphage MS2	19	92±10	4	71±25
Bacillus atrophaeus	18	61±11	4	55±12
No Significant Difference by Mann Whitney Test for *E. coli*, coliphage MS-2, and *Bacillus atrophaeus*; p values = 0.7846, 0.3657, and 0.8375, respectively.				

*All experiments performed with modified endcaps on F200A HFUFs

A Fresenius F200A with conventional endcaps was compared to a system using two HFUFs with conventional endcaps in series, based on flow rates for treated drinking waters. Average treated drinking water filtration rates through HFUFs with conventional endcaps as a single unit versus two in series were 0.57±0.14 L/min versus 1.25±0.24 L/min. This average filtrate rate difference of 0.68 L/min., which is more than twice that of a single HFUF, was not quite statistically significant (Mann-Whitney Test; p value = 0.0702). Therefore, a single HFUF and two HFUFs in series had potentially different average

filtration rates, with potentially shorter treated drinking water sample processing times for the latter.

A single Fresenius F200A with modified endcaps was compared to using two HFUFs with modified endcaps in series, based on filtration rates for treated drinking water. Average filtration rates for the single and two in series HFUFs with modified endcaps were 0.66±0.03 L/min, and 1.62±0.19 L/min, respectively. The average filtration rate difference of 0.96 L/min. was statistically significant (Mann-Whitney Test; p value = 0.0009), demonstrating that two HFUFs in series had significantly higher average filtration rates and sample processing times compared to a single HFUF.

F. Effects of Water Type on Microbial Indicator Recoveries and Processing Times

Overall average microbial recoveries for source and treated drinking waters utilizing a single HFUF were compared for both SFPUC (Table 16) and OWASA waters (Table 17). For SFPUC waters, overall average microbial indicator recoveries from treated versus source water were 60±09% versus 83±44% for *E. coli* K011, 78±10% versus 83±21%, respectively, for coliphage MS-2, and 63±9% versus 62±12%, respectively, for *Bacillus atrophaeus*. These recoveries of *E. coli*, MS-2 and *Bacillus atrophaeus* from SFPUC treated and source waters were not statistically significance (Mann-Whitney Test; p values= 0.8758, 0.3682, and 0.8385, respectively). For OWASA waters overall average microbial indicator recoveries from treated versus source waters were 77±19% versus 129±29%, respectively, for *E. coli* K011, 92±10% versus 83±12%, respectively, for coliphage MS-2, and 61±11% versus, 151±27%, respectively, for *Bacillus atrophaeus*. *E. coli* and coliphage MS-2 recoveries based on water type were no statistically significantly different (Mann Whitney Test; p

values = 0.2886 and 0.7204, respectively), but *Bacillus atrophaeus* spore recoveries were statistically significance different (Mann Whitney Test; p value = 0.0116), with higher recoveries from source waters. These results suggest that water type does not affect microbial indicator recoveries using a single HFUF for *E. coli* and coliphage MS-2 data but it does influence recoveries of *Bacillus atrophaeus* spores

Table 16. Overall Recovery of Microbial Indicators from Source and Treated Drinking Waters using a Single HFUF System in SFPUC Waters

Organism	Treated		Source	
	Trials (N)	Average Recovery (%)	Trials (N)	Average Recovery (%)
E. coli K011	26	60±09	10	83±44
Coliphage MS2	26	78±10	8	93±21
Bacillus atrophaeus	26	63±09	9	62±12
No Significant Difference by Mann Whitney Test for *E. coli*, coliphage MS-2, and *Bacillus atrophaeus* spores from source waters compared to treated drinking waters. (Mann-Whitney Test; ; p values = 0.8758, 0.3682 , and 0.8385 respectively)				

*All experiments performed with modified endcaps on both HFUF designs

Table 17. Overall Recovery of Microbial Indicators from Source and Treated Drinking Waters using a Single HFUF System in OWASA Waters

Organism	Treated		Source	
	Trials (N)	Average Recovery (%)	Trials (N)	Average Recovery (%)
E. coli K011	19	77±19	12	123±29
Coliphage MS2	19	92±10	13	83±12
Bacillus atrophaeus	18	61±11	12	151±27
No Significant Difference by Mann Whitney Test for *E. coli* and coliphage MS-2; p values = 0.0848 and 0.6498 respectively. Significantly higher recoveries of *Bacillus atrophaeus* spores from source waters compared to treated drinking waters (Mann-Whitney Test; p value = .0116)				

*All experiments performed with modified endcaps on both HFUF designs

VI. DISCUSSION

Waterborne outbreaks of infectious diseases continue to threaten human life with no indication of elimination in the near future by current control measures. Methods have been developed and accepted for concentration of individual classes of microbial pathogens from water, such as EPA Method 1623 for *Cryptosporidium* and *Giardia* and the so-called Information Collection Rule (ICR) Virus Method which utilizes a charge-modified electropositive cartridge filter. Neither of these methods are suggested for rapid detection, nor can they be used for simultaneous concentration of multiple classes of pathogenic organisms, as may be needed for response to emergency situations, such as bioterrorism events or natural contamination such as weather related flooding. Methods have been described in the literature that use hollow-fiber ultrafiltration for simultaneous and reliable concentration of multiple classes of microorganisms from different water types (Klein, Mahlandt et al. 1971; Cheryan 1986; Kuhn and Oshima 2001; Simmons, Sobsey et al. 2001; Kuhn and Oshima 2002; Morales-Morales, Vidal et al. 2003). Therefore, hollow-fiber ultrafiltration has been suggested as a potential candidate for use in emergency response for concentrating multiple classes of microorganisms from different water types. It may also be useful for routine monitoring where its low cost would be superior to current methods that more microbe-specific and more expensive. However, a major criticism of this method continues to be long sample processing times which detract from it being a "rapid" method as well as a limiting factor for using this technique for the large sample volumes necessary to lower method detection limits. These obstacles must be addressed if hollow-fiber

ultrafiltration is to become a preferred method for detection of microorganisms in water and are in this research with the hydraulic modifications of the HFUF through endcaps and larger tubing as well as using more than one HFUF in multiple combinations to allow for an increased filtrate rate with no reduction in microbe recovery rate.

It is noteworthy that many of the calculated recovery efficiencies of this study are greater than 100%, which intuitively should not be possible. A review of similar microbial recovery trials reported in the literature, indicates that this is not an uncommon occurrence, especially for environmental waters that may have naturally occurring background microbe levels. Other explanations for these high recoveries include bias in the estimates of initial test microbe levels in test waters, variability between analysts and laboratories, assay variability, and variability in the properties of the microbe stocks used. Underestimates of initial microbe levels in test waters, due to aggregation, or underestimates of recovered microbes levels, such as due to interferences with microbe assay methods can cause such overestimates of microbe recoveries in excess of 100%.

The water type may also play a critical role as a source of variability in microbe recovery. Because, source and treated drinking water have inherently different physical and chemical characteristics, organisms suspended in each water type may behave differently to concentration and assay methods. A robust concentration method is applicable to a variety of water types, which necessitates assessment of the method for recoveries from both source and treated drinking water samples. One example of effects attributable to the chemical and physical quality of the water would be the potential for microbial aggregation or association with particulate matter in source waters due to a higher organic content and variable pH, particle association, aggregation and pH influence microbial recovery efficiency and microbe

responses to concentration methodologies. Treated drinking waters tend to have a more constant pH and less particulate and organic matter, which may favor microbial recovery and detection. However, treated waters have other properties, such as disinfectant residual, that need to be accounted for. Because of these dissimilarities in water type, the two water types, (source, treated) were addressed separately in these experiments. Many experiments were done with paired water samples of specified quality such that any variability in water type or geographical region was internally controlled. The methods for concentrating microbial indicators and pathogens from water are adaptable and can be applied to treated drinking water samples in a central facility or to source waters that are processed in the field. The observed differences in water type may directly influence the equipment needs for implementing these methods in the future.

Microbial indicator recoveries for each water type were relatively high and consistent. The exception was *Bacillus atrophaeus* spores, which showed a significant difference in recovery efficiencies between the two types of water studied (Table 17). Possible explanations for these recovery differences may be attributable to specific organism characteristics, unknown matrix effects associated with the physical or chemical quality of the test waters, or methods of spore assay.

Each of the three test microorganisms represents a different microbial class with different surface properties, morphology, and size. Such differences may account for the observed differences in recovery of the *Bacillus atrophaeus* spores when compared to the other test microorganisms. *Bacillus atrophaeus* is a spore forming, Gram positive bacterium, *E. coli*, is a Gram negative, non spore forming bacterium and coliphage MS-2 is a male-specific RNA coliphage of the family *Leviviridae* which is very small compared to bacteria. The Gram

positive bacteria, such as *Bacillus atrophaeus*, typically have a smoother surface with a smaller variety of proteins, which typically results in less aggregation or particle association when compared with Gram negative bacteria, such as *E. coli*.(Madigan, Martinko et al. 2006) The cell wall of Gram positive bacteria consists of 90% peptidoglycan, with small amounts of teichoic acid. This composition determines the overall shape and smoothness of the bacterium giving it a more rigid structure and layering (Madigan, Martinko et al. 2003). For a spore forming organism, however, the spore state has other properties. Previous studies with *Bacillus anthracis* document aggregation, as well as surface hydrophobicity which greatly contributed to the aggregation (Wang, Gu et al. 2006). For coliphage MS-2 (approximately 27-34 nm in diameter), size is an important factor related to concentration by recovery methods. In comparison *Bacillus atrophaeus* is 300-500 nm in size. Therefore, variable recovery efficiencies may be attributable to differences in microbe class and to quality differences in water types, such as in their types and concentrations of particulate matter and organic matter.

Indicator organisms and not frank pathogens were used in this study because they are safer, easier and timelier to culture, and they have proven reliable for modeling pathogens for more than 100 years in the case of bacteria. The use of microorganisms representative of each pathogen class was considered necessary. Therefore three indicator organisms were chosen: *E. coli* (bacteria), coliphage MS-2 (viruses), and *Bacillus atrophaeus* (bacterial spores, considered somewhat representative of protozoan parasites).

A primary goal of these experiments was to decrease sample processing times while maintaining efficient recovery efficiency for multiple classes of microbial indicators in source and treated drinking water samples. It was hypothesized that as the flow rate increased

within the hollow-fiber ultrafiltration system, the performance of the system would also increase, resulting in decreased sample processing times. In general, the microbial recovery efficiencies remained consistently high for all classes of indicators in these trials, regardless of hydraulic conditions and filter configurations tested. It is noteworthy that there was no microbial breakthrough in HFUF filtrates (data not shown), which is consistent with results of previous experiments (Hill, Polaczyk et al. 2005).

Three hydraulic configurations of HFUFs were studied and compared for performance: modified endcap designs that increased cross-sectional surface area for influent water, different HFUF models with different tubule designs for different filter surface area within the HFUF, and use of two HFUFs in series or parallel to provide increases in filter surface area.

Sample processing times for both water types decreased when the HFUF with modified endcap design was used. Filtration rates increased by 10% in source water and by 171% in treated water. Filtration rate increases with the modified endcaps were statistically significantly higher than filtration rates with the conventional endcaps in the treated drinking water samples but not in source water samples. In general, endcap modifications allowed for a faster sample processing time without loss in microbial recovery efficiency.

HFUFs with different tubule design were available from Fresenius Medical Care AG & Co. KGaA Bad Homburg, Germany: the Fresenius HemoflowTM F80A and the Fresenius Optiflux® F200A. The surface area within the F200A was higher due to tubule design modifications. Briefly, the F80A has a basic straight tubule design as compared to the more torturous path design of the F200A. This design difference equates to a 2.0 m^2 increase in surface area within the HFUF system. Statistical analyses of the data from all recovery trials

comparing these two filters showed that there was no significant difference in microbe recovery efficiencies, from source waters.

Two HFUFs were used in an attempt to further increase the filtering surface area within the water processing system and thereby increase the filtration rate and reduce sample processing times. Two HFUFs were in parallel as well as in series. Two HFUFs in series consistently performed better than either the single HFUF or two HFUFs used in parallel. However, it should be noted that there were pressure differences within each system, with lower pressures in the parallel configuration than in the series configuration. Because pressure was not controlled in these experiments, further research is recommended to examine the effects of increased pressure on filtration rate and microbe recovery in the parallel configuration. Each of these HFUF hydraulic configurations was equivalent and not statistically different for recovering microbial indicators from water samples, using both the conventional and modified endcaps. However, filtration rates were increased for several experimental conditions.

In source waters two Fresenius F80A filters with conventional endcaps and operated in parallel produced higher (faster) filtration rates than a single HFUF with conventional endcaps. For the same experiment with HFUFs having modified endcaps, filtrate rates were equivalent, and not statistically different. However, the system using two Fresenius F80A HFUFs in series showed 169% and 207% increases in filtration rate over the single HFUF system with the standard endcaps and the modified endcaps, respectively. These increased filtration rates for two HFUFs in series were statistically different. Therefore, this modification significantly reduced sample processing times over use of a single HFUF.

In treated drinking waters two Fresenius F200A HFUFs with modified endcaps also showed superior performance for microbial recovery and reduced sample processing times. Two HFUFs with conventional endcaps, run either in parallel or in series, did not show significant increases in the filtration rates over the single HFUF system. However, the system of two HFUFs with modified endcaps operated in parallel showed a 77% increase in filtration rate over the system using a single HFUF. Two HFUFs with modified endcaps and operated in series showed a 145% increase in filtration rate over a single HFUF. Both two filter HFUF systems gave statistically significant increases in filtration rates compared to a single HFUF.

Three eluting solutions were tested to recover concentrated microbial indicators from the HFUF system when applied source or treated drinking waters. Eluting solution 1 was superior to the other two based on ease of preparation and all 3 gave comparable microbial recoveries. Eluting solution 1 was used exclusively for experiments to test hydraulic configurations of to the HFUF system. Each of the eluting solutions contained a surfactant as one of the primary ingredients. The use of surfactants has been well documented in the literature for recovery of microbial indicators and pathogens from concentration systems (Simmons, Sobsey et al. 2001; Sobsey, Yates et al. 2004). Surfactants generally work to disperse particulates in a solution and to reduce non-specific binding of microorganisms to surfaces, such as to the inside walls of the tubing and to the hollow fiber tubules within the HFUF system. The results of this study support previous literature concerning eluting solutions, which report variable recovery rates based on eluting solution tested, The main constituents of value within most eluting solutions are buffers that maintain microbe health and surfactants that prevent microbe attachment to tubing during ultrafiltration process

(Kuhn and Oshima 2001; Kuhn and Oshima 2002; Morales-Morales, Vidal et al. 2003; Hill, Polaczyk et al. 2005).

Filtration rates of this study cannot be directly compared to those of previous studies due to the unique use of both the modified endcaps and two HFUFs in parallel or in series in an effort to produce a more rapid filtration system. However, microbe recovery rates are clearly documented in previous published studies and they support the reliability of this system. Source (181%) and Treated (112%) recovery rates of an antibiotic resistant strain of *E. coli* were shown to be relatively consistent with previously published literature, with *E. coli* recovery of 97% from Hetch Hetchy waters (Morales-Morales, Vidal et al. 2003) 70-84% from sterile waters(Polaczyk, Narayanan et al. 2008).The recoveries of coliphage MS-2 and *Bacillus atrophaeus* in these trials also show consistency with previous literature. Recovery rates ranged from 92%-106% for MS-2, and 57-165% for *Bacillus atrophaeus* in these trials, which are comparable to recovery rates of 78-85% and 49-53%, respectfully, in similar waters of previous studies (Polaczyk, Narayanan et al. 2008)

In conclusion, these results demonstrate the effectiveness of hollow-fiber ultrafiltration. Systems with modified endcaps and the use two HFUFs in series for efficient and more rapid concentration of multiple classes of microorganisms from source and treated drinking waters. Each of these modifications increases the flux of the system while maintaining overall microbial recovery efficiency. This increase in flux effectively reduces the time for sample processing which has been a major limitation for the use of hollow-fiber ultrafiltration for concentrating microbial indicators and pathogens from large volume water samples. Larger samples can now be processed in the field as well as in the laboratory in a more rapid manner utilizing this one method to concentrate multiple classes of microorganisms. This system

allows for a more rapid response in the event of a water contamination incident or threat and results in a more cost efficient method to replace the current multiple and more expensive microbial concentration methods now in use.

VII. CONCLUSIONS

The Main conclusions from this research are:

- Hollow-fiber ultrafiltration using sterile, disposable Fresenius cartridges can concentrate multiple classes of microbial indicators from source and treated drinking water samples,
- Water type, either source or treated, had no effect on the ability to concentrate bacteria and viruses; However; bacterial spores were concentrated more effectively from source water compared to treated drinking water,
- Microbial recovery efficiency is reliable, consistent, and robust for HFUF filtration, regardless of hydraulic modifications the hydraulic configurations of the HFUF system, tested, namely single cartridge or two HFUF cartridges used either in series or in parallel. Recoveries averaged 79±09, 86±06, and 90±10% for *E. coli*, MS-2, and coliphage spores of *Bacillus atrophaeus* respectively, for both water qualities and all filters and filter configurations combined,
- Recovered microbial indicators were detectable by infectivity assays, which documents that recovered microbes were not inactivated by HFUF concentration procedures
- Of the three eluting solutions tested, all showed high and statistically equivalent recovery efficiencies for all three microbial indicators concentrated by the HFUF system,

- When two HFUFs that differed in tubule design (Fresenius F80A and F200A) were compared in source waters they showed statistically comparable microbe recoveries and filtration rates.
- A hollow-fiber ultrafiltration system utilizing modified endcaps for increasing the cross-sectional area of flow to the HFUF effectively increased the flux of water to the filter and resulted in higher filtrate rates and reduced sample processing times,
- A hollow-fiber ultrafiltration system utilizing two HFUFs in series increased the filtering surface area and resulted in significantly reduced sample processing times, and
- A hollow-fiber ultrafiltration system using two HFUFs with modified endcaps in series resulted in higher flow rates significantly and reduced sample processing times.

VIII. FUTURE RESEARCH

Future research that should be considered includes:

- Design modifications should be made to the system using two HFUFs in parallel such that water is plumbed to each HFUF at a higher pressure,
    - This could be accomplished by using two peristaltic pump-heads for each HFUF, effectively splitting the flow at the water reservoir within the system,
- The HFUF system should be tested for compatibility with microbial pathogens of concern to demonstrate consistent results to those achieved with microbial indicators,
- Given decreased sample processing times achieved by the hydraulic modification described, larger sample volumes should be processed to demonstrate scalability of the system,
- HFUF concentrates should be subjected to secondary concentration and isolation procedures, such as immunomagnetic separation, polyethylene glycol precipitation, and organic flocculation to demonstrate compatibility,
- Compatibility with rapid molecular detection methodologies should be demonstrated in order that this be accepted as a "rapid" method for emergency response,
- Hollow-fiber ultrafiltration should be tested for use in concentrating other classes of contaminants, such as chemical contaminants and biological toxins,
- A Standard Operating Protocol (SOP) should be developed that can be round-robin tested to demonstrate technology transfer to local, State, and Federal laboratories;

- A field portable system should be developed that can be deployed for emergency response or for routine primary concentration of microbial indicators and pathogens in the environment,
- Information gained from this more robust and reliable method for recovering microbial pathogens in the environment should be used in Quantitative Microbial Risk (QMR) models to better identify risks from source and treated drinking waters thereby providing a greater measure of public health

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